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THE FEEDING VALUE OF GRASS SILAGE IN THE RATION FOR DAIRY COWS

O. L. LEPARD¹ AND E. S. SAVAGE

Department of Animal Husbandry, Cornell University, Ithaca, New York

The ensiling of legumes and grasses has been practiced to a limited extent for years, although the practice has not been generally accepted because of recurrent failures. Recent investigations have shown possible methods of preventing these failures. The ensiling of legumes and grasses by these methods has been spurred on during recent years by extension workers, interested in the production of better quality roughage, and by commercial companies, interested in the sale of preservatives or equipment used in the process of ensiling.

The terminology with reference to ensiled crops is not uniform. Throughout this report the terminology of Bender and Savage (2) will be used. They used the term "grass silage" as an all-inclusive term referring to any "silage made from an uncured hay crop—whether it be a true grass such as timothy, a legume such as alfalfa, or a green cereal such as oats. Terms like 'alfalfa-molasses silage' or 'timothy-phosphoric acid silage' describe specific kinds of grass silage."

Among the feeding experiments with grass silage, Watson and Ferguson (15) have made extensive reports. They fed respective groups of dairy cows A.I.V. silage, molasses silage, and artificially dried grass. A statistical analysis of the milk production, butterfat production, and live weight changes showed no significant difference when equal amounts of starch equivalent and digestible crude protein were furnished in each ration.

A comparison of alfalfa silages prepared by the A.I.V. and molasses methods was made by Bohstedt *et al.* (3) in which feeding trials were carried out for three years on milking animals. During the three years the milk and butterfat production records were maintained almost equally well, although the molasses silage had a slight advantage. Each animal received 1 oz. of CaCO_3 - Na_2CO_3 mixture (10:3) per 15 pounds of silage.

Bender *et al.* (1) have shown that molasses grass silage will replace corn

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¹ Now Associate in Dairy Husbandry at the New Jersey Agricultural Experiment Station, Sussex, New Jersey.

silage or hay in the ration of a dairy cow without influencing the production level of the cows to a marked extent. When fed in place of both corn silage and hay, the production level was maintained, although there was a slight loss in body weight. It was believed that a slight increase in the grain ration could have overcome the slight loss in body weight manifested by the animals fed grass silage as the only roughage.

Alfalfa hay grown on irrigated land was compared with mixed grass and clover hay and grass silage by Hodgson and Knott (6). They fed a concentrate mixture according to production. The cows on the mixed hay and silage ration consumed 78.6 per cent as much dry matter as the alfalfa hay group. The cows fed alfalfa hay lost only 33 per cent as much live weight as the cows receiving the mixed hay and grass silage ration. The production of milk for the cows on the mixed hay and grass silage ration was 94.2 per cent as much as for those on the alfalfa ration.

Hayden *et al.* (5) fed two groups of milking cows A.I.V. alfalfa silage and alfalfa hay respectively. By a reversal method they found no significant difference in the production of the animals in the two groups. The A.I.V. silage was supplemented with four ounces of ground limestone per cow per day.

Many other feeding experiments (8, 11, 12, 13, 14) have been conducted to determine the relative value of different silages, different plants in dry or ensiled form, and different combinations of these plants. These experiments were usually of short time duration and, almost without exception, showed no significant difference between the various feeds when they were of good quality and the dry matter intake of the animal is constant.

The work here reported from the Cornell University Experiment Station covers four feeding experiments with milking animals. These experiments were designed to compare grass silage preserved with phosphoric acid with grass silage preserved with molasses, and to determine their value in replacing varying amounts of corn silage and dry hay.

The general procedure for all experiments included complete feed and production records. The animals were milked and fed twice daily. The gain or loss of body weight was determined from the average of three daily weights of the cows, taken at the beginning and end of each period in all experiments.

The chemical analyses of all feeds and the digestion coefficients used in calculations are given in tables 4 and 5. The analysis of the grain mixture was computed from Morrison's tables (9). This computed analysis was used in determining the consumption of feed nutrients in the grain mixture.

EXPERIMENT I

In this experiment the basic ration of one pound of dry hay and three pounds of corn silage per hundred pounds of body weight was varied by the

replacement of a part of the corn silage or dry hay, or both, with grass silage, and the effect on production and body weight determined. A triple reversal experiment was planned with five milking animals in each of three groups, A, B, C. The groups were balanced as closely as possible in milk production, fat test, body weight, breed, age, date bred, and condition.

The three rations used were as follows:

Ration I:

Corn silage—3 pounds for each 100 pounds live weight.

Mixed hay—1 pound for each 100 pounds live weight.

Grain—an 18 per cent concentrate mixture according to production.

Ration II:

Corn silage—3 pounds for each 100 pounds live weight.

Mixed hay— $\frac{1}{2}$ pound for each 100 pounds live weight.

Molasses silage (grass, clover and alfalfa)—2 pounds (equivalent to $\frac{2}{3}$ pound of mixed hay) for each 100 pounds live weight.

Grain—an 18 per cent concentrate mixture according to production.

Ration III:

Corn silage—2 pounds for each 100 pounds live weight.

Mixed hay— $\frac{2}{3}$ pound for each 100 pounds live weight.

Molasses silage (grass, clover and alfalfa)—2 pounds (equivalent to $\frac{2}{3}$ pound mixed hay) for each 100 pounds of live weight.

Grain—an 18 per cent concentrate mixture according to production.

The corn silage was well-eared and at a medium stage of maturity.

The grass silage was preserved with 41.75 pounds of blackstrap feeding molasses per ton of green crop. The approximate per cent of each crop in the silage was as follows: grasses—40 per cent; clovers—30 per cent; alfalfa—20 per cent; weeds—10 per cent. The crop was cut early and stored in the silo with a minimum of drying. After the usual spoilage at the top, the rest of the silage came out in excellent condition.

The dry hay was mixed grasses, clovers, and alfalfa of good quality, green and early cut. It was unofficially graded as U. S. No. 1 mixed hay. It was selected as being comparable to a type of hay that could have been made from the green crop used for grass silage under desirable weather conditions.

The grain used was a regular 18 per cent protein commercial open formula mixed feed as follows:

480	lbs.	corn	gluten	feed
200	"	wheat	bran	
100	"	hominy	feed	
220	"	ground	oats	
220	"	coconut	oil	meal
300	"	corn	distillers	dried grains

100	"	soybean oil meal (hydraulic or expeller process)
160	"	ground barley
180	"	cane molasses
20	"	steamed bone meal
20	"	common salt

The three rations were fed to the respective groups in the following sequence:

	<i>Ration I</i>	<i>Ration II</i>	<i>Ration III</i>
First 5-week period	Group A	Group B	Group C
Second 5-week period	Group C	Group A	Group B
Third 5-week period	Group B	Group C	Group A

In determining results the feed consumed and milk produced were totalled from the three groups on each ration and reduced to the basis of one cow for one day. These final data are given in table 1.

This table indicates that the production of the animals on the different types of rations was practically the same. The greatest difference in daily average production of 4 per cent fat-corrected milk was 1.2 pounds. This is not a significant difference. The animals on Ration I had a slight advantage in that they received more total digestible nutrients. This caused a gain in weight of the animals on this ration.

EXPERIMENT II

The effect of continuous feeding of molasses grass silage as the only roughage was studied. Ordinary dairy practices call for both a succulent and a dry feed, and it is usually supposed that a combination of feeds is advantageous over a single feed.

A continuous 12-week feeding experiment was planned. Eleven animals were selected which were in fairly heavy milk production, but had passed their peak of lactation. All animals were less than three months pregnant at the end of the experiment. All of the animals were in good and thrifty growing condition. Animals of various breeds and body weights were used in the experiment.

The maximum amount of molasses silage (grass, clover and alfalfa) that the animals would consume was fed. The body weights and the condition of the animals were very closely watched and enough grain was fed to maintain both normal milk production and body weight. It was assumed that the animals' milk production should not decline faster than a normal production curve, and that they should gain weight slightly during advancing lactation, or at least maintain weight during the period of this experiment.

Molasses silage (grass, clover and alfalfa) was fed from the same silo as in Experiment I. Each animal was fed as much of this silage as it would consume. The grain was of the same mix as that used in Experiment I. An attempt was made to feed it in such quantities that the milk production and body weights be maintained in a normal way.

TABLE 1

Daily average feed consumption, nutrient consumption, weight change, and production of animals on the various rations of Experiment I, II, III, and IV

Ration	Grain	Dry hay, lb.	Molasses grass silage, lb.	Phosphoric grass silage, lb.	Corn silage, lb.	T.D.N. fed, lb.*	Digestible protein fed, lb.*	Milk produced, lb.	Butterfat percentage	Fat produced, lb.	Production % fat-corrected milk, lb.†	T.D.N. required for maintenance and production†	Digestible protein required, lb.‡	Excess T.D.N.	Excess lb. digestible protein
Daily average per cow for Experiment I (3 periods)															
.....	7.7	11.2	33.4	16.4	2.22	26.5	3.66	.97	25.1	16.9	1.95	-.5	+.27
.....	7.6	3.7	21.1	33.1	14.9	2.06	26.7	3.70	.99	25.5	17.1	1.97	-2.2	+.09
.....	7.9	7.3	20.9	22.2	15.6	2.20	28.0	3.58	1.00	26.3	17.3	2.01	-1.7	+.19
Daily average per cow for Experiment II															
.....	16.44	71.77	21.33	3.60	43.04	3.83	1.05	41.96	22.82	2.81	-1.49	+.79
Daily average per cow for Experiment III (2 periods)															
.....	10.2	28.7	33.9	19.7	3.1	28.8	4.26	1.23	29.93	18.4	2.2	+1.4	+.9
.....	10.2	32.9	32.3	19.4	2.5	27.9	4.33	1.21	29.32	18.2	2.1	+1.2	+.4
Daily average per cow for Experiment IV (2 periods)															
.....	13.87	10.28	37.32	22.6	3.7	36.9	4.13	1.52	37.58	20.4	2.5	+2.3	+1.1
.....	13.78	9.81	29.49	21.9	4.1	36.6	4.09	1.49	37.06	20.7	2.5	+1.2	+1.6

Calculated from average of analyses made during experiment (tables 4 and 5).

Calculated according to Gaines (4).

Calculated according to recommended standards by Morrison (9) for each animal and totalled.

Weight used was average of initial and final weights for period.

Table 1 gives records of the feed consumed, nutrients consumed, weight change, and production of the animals on Experiment II. This shows clearly that a very constant weight was maintained. This, however, was accomplished with a rather high rate of grain feeding. This high rate of grain feeding was necessary in order to make up for the lack of total digestible nutrients consumed in the roughage part of the ration.

Because this was a continuous feeding experiment with no control group, the production of these animals must be compared with normal.

Landis (7), working with Savage, plotted 389 lactations of animals from three large herds. These animals gave, on the average, a 2 per cent decline per week between the peak of lactation and the fifth month following breeding. Because these records were largely taken from experiment station herds, the normals derived should be comparable to those used in the experiment.

Table 2 gives the average daily production for each week of the 11 animals, and their calculated production (with a normal 2 per cent per week decline) according to Landis and Savage. It is indicated clearly in table 2 that the production of animals on the continuous feeding of molasses silage (grass, clover and alfalfa) was maintained during the duration of this experiment.

TABLE 2

A comparison between the actual production and normal expectancy of production per cow per day for animals on experiment II, on continuous feeding of molasses silage (grass, clover and alfalfa) as the only roughage

Week	Actual daily production*	Actual per cent decline	Expected normal daily production†
Initial	44.55	44.55
1	44.29	0.6	43.66
2	45.97	- 3.8	42.79
3	45.76	.5	41.93
4	43.81	4.3	41.09
5	43.19	1.4	40.17
6	42.73	1.1	39.37
7	41.68	2.4	38.58
8	39.97	4.1	37.81
9	40.82	- 2.1	37.05
10	38.89	4.7	36.31
11	38.26	1.6	35.58
12	38.26	0.0	34.87

* Four per cent fat-corrected milk calculated according to Gaines (4).

† Calculated according to Landis (7) (2 per cent decline per week).

As this experiment was set up for ad libitum consumption of grass silage, it is significant to note the variation in consumption of grass silage by the different animals. This is shown as daily consumption of grass silage per animal per 100 pounds of live weight. These data, with the grain to milk ratio, are given in table 3 for each animal.

It is evident from the data in this table that the rate of consumption

among individual animals is very variable, and that, when the consumption per 100 pounds of body weight decreases, the grain to milk ratio must increase inversely in order to supply the necessary feed nutrients.

TABLE 3

Illustrating high grain feeding necessary to maintain production and body weight on a continuous feeding of molasses silage as the only roughage

Breed	Lb. grass silage consumption per 100 lb. live weight	Lb. of 4 per cent fat-corrected milk produced daily*	Lb. of 4 per cent fat-corrected milk produced for each lb. of grain fed*
Holstein	6.61	57.90	2.84
Ayrshire	6.02	36.49	2.38
Holstein	5.29	54.14	2.58
Holstein	6.13	38.35	2.26
Jersey	5.64	33.05	2.34
Holstein	3.87	48.99	2.13
Holstein	6.18	40.77	2.71
Ayrshire	4.68	29.82	2.21
Holstein	7.91	38.43	2.38
Guernsey	7.63	38.92	3.31
Shorthorn	5.96	44.75	3.25
Actual average ...	5.99	41.96	2.55

* Calculated according to Gaines (4).

EXPERIMENT III

The use of phosphoric acid was proposed for the preservation of green grass by Wilson (16). Because of the widespread publicity given to this method by commercial companies, and its adoption by many farmers, a single reversal feeding trial was planned to compare the value of a green crop preserved with molasses and one preserved with phosphoric acid when both were fed with corn silage and no hay.

The twelve milking animals selected for this experiment were divided into two balanced groups.

The rations used for this group of animals are designated as Rations I and II. They are as follows:

Ration I:

Corn silage—3 pounds for each 100 pounds live weight.

Molasses silage (grass, clover and alfalfa)—3 pounds (equivalent to 1 pound mixed hay) for each 100 pounds live weight.

Grain—an 18 per cent concentrate mixture according to production.

Ration II:

Corn silage—3 pounds for each 100 pounds live weight.

Phosphoric acid silage (grass, clover and alfalfa)—3 pounds (equivalent to 1 pound mixed hay) for each 100 pounds live weight.

Grain—an 18 per cent concentrate mixture according to production.

The corn silage was made from a crop that was well-eared and at a medium degree of maturity.

The molasses grass silage was preserved with 48.06 pounds of blackstrap feeding molasses per ton of green crop. The approximate per cent of each crop in the silage was as follows: grasses, 35; clovers, 40; alfalfa, 20; weeds, 5. The crop was cut early and stored in the silo with a minimum of drying. It was, however, felt that water would increase the value of the silage. Therefore, a three-fourth-inch stream of water was entered at the throat of the chopper. After the usual spoilage at the top, the rest of the silage came out in excellent condition.

The phosphoric acid grass silage (grass, clover and alfalfa) was preserved with 27.5 pounds of 68 per cent (food grade) phosphoric acid per ton of green material. The approximate per cent of each crop in the silage was as follows: grasses, 50; clovers, 25; alfalfa, 20; weeds, 5. The crop was cut early and stored in the silo with a minimum of drying. Water was added to this grass silage as it was to the molasses grass silage with a three-fourth-inch stream at the throat of the chopper. There was considerable spoilage at the top and around the edges through the entire depth of the silage. This was believed to be due to air leakage through a poorly constructed silo wall. Only good silage was selected and used to feed the experimental animals.

The grain mixture fed to both groups was a special 18 per cent protein, commercial, open formula mixed feed, as follows:

400	lbs. wheat bran
400	" ground corn or hominy
370	" ground oats
300	" fresh coconut oil meal
300	" corn distillers' dried grains (not less than 10 per cent fat)
200	" linseed oil meal, O.P.
20	" steamed bone meal
10	" common salt

In determining results the feed consumed and milk produced were totalled from the two groups when on each ration and reduced to the basis of one cow for one day. These final results are given in table 1.

These indicate that, with the conditions as represented in this experiment, there was little difference between the two rations. That is, grass silages preserved with molasses and grass silages preserved with phosphoric acid were equal in value as a supplement to corn silage.

EXPERIMENT IV

This experiment was designed much the same as Experiment III. Grass silage preserved with phosphoric acid was fed to one group, and grass silage preserved with molasses was fed to another group. The one important dif-

ference is that dry hay was used to supplement the grass silage, instead of corn silage. The object of the experiment was to compare the value of the two silages when fed with dry hay.

The ten milking animals selected for this experiment were divided into two balanced groups, A and B.

The rations were as follows:

Ration I:

Mixed hay—1 pound for each 100 pounds live weight.

Phosphoric acid silage (grass, clover and alfalfa)—3 pounds (equivalent to 1 pound mixed hay) for each 100 pounds live weight.

Grain—an 18 per cent concentrate mixture, according to production.

Ration II:

Mixed hay—1 pound for each 100 pounds live weight.

Molasses silage (grass, clover and alfalfa)—3 pounds (equivalent to 1 pound mixed hay) for each 100 pounds live weight.

Grain—an 18 per cent concentrate mixture according to production.

The mixed hay was early cut and well cured. It was unofficially graded as follows: U. S. Grade No. 2 mixed hay. It contained timothy, red top, quack, alfalfa, blue grass, and red clover. Both the molasses and phosphoric acid grass silage were from the same silos as that used in Experiment III. The grain used was of the same mix as that used in Experiment III.

In determining results the feed consumed and milk produced were totalled from the two groups when on each ration and reduced to the basis of one cow for one day. These data are given in table 1.

These indicate that, with the conditions as represented in the experiment, there was little difference between the two rations. That is, grass silage preserved with molasses and grass silage preserved with phosphoric acid were equal in value as a supplement to mixed hay.

DISCUSSION OF RESULTS

Throughout these feeding experiments it was evident that the palatability of the silage depended on the quality of the crop ensiled, moisture content, and the preservative added.

These experiments indicate clearly that the production of the animals was not significantly different on the various combinations of roughage, providing the total nutrient intake remained constant.

When molasses grass silage was fed as the only roughage ad libitum in Experiment II, the consumption of nutrients in the form of roughage varied considerably with individuals. The average was 5.99 pounds of grass silage per hundred pounds, which furnished nearly the normally expected intake of nutrients in the form of roughage. The range, however, was from 3.87 to 7.91 pounds per hundred pounds live weight, which means that many animals will not consume so large an amount of dry matter in the form of

molasses grass silage as in combinations of more than one roughage as a succulent and dry feed. This necessitates the feeding of a greater amount of concentrates. In this regard it is significant to note that there was a great range in the rate of grain feeding. Certain animals consuming a small amount of grass silage were fed on a narrow grain ratio (as narrow as one pound of grain to 2.13 pounds of 4 per cent fat-corrected milk), while the others consuming a large amount of grass silage were fed on a wider grain ratio (as wide as one pound of grain to 3.25 pounds of 4 per cent fat-corrected milk). The average was one pound of grain to 2.55 pounds of 4 per cent fat-corrected milk. This was the narrowest grain to milk ratio of any group of animals included in the feeding experiments.

Although the production and body weights were satisfactorily maintained on a continuous feeding of molasses grass silage as the only roughage, it would not seem a practical feeding practice because of the decreased nutrient consumption in the form of roughage.

From Experiments III and IV there is evidence that the kind of preservative did not affect the feeding value of the silage, providing the total intake was maintained. There is also evidence to indicate that either phosphoric acid or molasses grass silage is a satisfactory supplement to corn silage or dry hay when fed under conditions as represented in these experiments.

When grass silage is used to replace all the dry hay, the ration may be deficient in vitamin D, and precautions should be taken against this deficiency when this feeding practice is continued over long periods. Similarly, when grass silage is used to replace corn silage, the nutrient intake may be lowered because of the lower percentage of total digestible nutrients in grass silage as compared with corn silage. This must be compensated for by increased consumption of either roughage or grain.

Grass silage usually contains a larger amount of protein than corn silage and, when used to replace corn silage, the protein content of the grain mixture may be lowered accordingly.

It must be remembered that grass silage is a variable product because of its moisture content, the crop from which it is made, and the manner in which it has been preserved.

SUMMARY AND CONCLUSIONS

Various combinations of molasses silage (grass, clover and alfalfa), corn silage and dry hay were fed to three groups of cows over a fifteen-week period. There was no significant difference in the production of the animals in the various groups, providing the total nutrient intake remained constant.

Molasses silage (grass, clover and alfalfa) was fed as the only roughage to eleven milking cows for twelve weeks. They maintained normal milk production and body weight. However, many of the animals were unable to consume the normal roughage nutrient intake on this diet. The decrease

in nutrient consumption was compensated for by an increase in the amount of grain.

In a ten-week single reversal feeding trial, phosphoric acid silage (grass, clover and alfalfa) was found to be equal in feeding value for milk production and maintenance of body weight to molasses silage (grass, clover and alfalfa) when the grass silages were used to replace corn silage in a normal ration of corn silage, mixed hay and grain.

TABLE 4
Chemical analysis of feeds used in experimental trials

Feed	Moisture	Protein	Ether extract	Crude fiber	Ash	Nitrogen free extract	Used in experiments
Corn silage	84.78	1.33	0.49	3.48	0.78	9.14	I
Corn silage	81.03	1.80	0.51	4.58	1.24	10.84	I
Corn silage	71.59	2.39	0.87	7.21	1.78	16.16	I
Molasses silage	80.25	1.36	0.53	6.27	1.01	10.58	I, II
Molasses grass silage	83.92	1.85	0.67	4.87	1.06	7.63	I, II
Molasses grass silage	74.52	2.76	1.14	7.68	1.58	12.32	I, II
Molasses grass silage	79.56	2.42	0.82	6.15	1.81	9.24	I, II
Mixed hay	5.41	8.74	2.02	28.87	5.96	49.00	I
Mixed hay	8.00	9.74	1.85	32.02	6.31	42.08	I
Mixed hay	6.21	10.91	2.30	28.52	6.81	45.25	I
Grain*	8.22	19.93	4.48	7.14	7.80	52.43	I, II
Corn silage	73.51	2.45	0.95	8.30	1.48	13.31	III
Molasses grass silage	65.41	6.33	1.18	11.08	2.90	13.10	III, IV
Phosphoric acid grass silage ..	70.21	2.71	0.80	11.24	2.00	13.04	III, IV
Grain*	9.77	19.37	5.40	8.94	6.24	50.28	III, IV
Mixed hay	8.27	15.34	1.76	35.02	7.39	32.22	IV

* Analyses according to Morrison (9) used in calculation.

In a twelve-week single reversal feeding trial, phosphoric acid silage (grass, clover and alfalfa) was found equal in feeding value for milk production and maintenance of body weight to molasses silage (grass, clover and alfalfa) when the grass silages were used to replace mixed hay in a normal ration of corn silage, mixed hay and grain.

TABLE 5
Digestion coefficients used (in per cent)

Feed	Protein	Ether extract	Crude fiber	Nitrogen-free extract	Experiment used	Reference
Corn silage	54.0	74.0	66.0	69.0	I, III	Morrison (9)
Grass silage	62.0	61.1	59.4	65.4	I, II, III, IV	Newlander <i>et al.</i> (10)
Mixed hay	59.0	58.0	51.0	69.0	I, IV	Morrison* (9)

* Used digestion coefficients for red clover hay all analyses.

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EFFECT OF PROTEOLYSIS ON LIPASE INDUCED RANCIDITY IN CHEDDAR CHEESE¹

I. HLYNKA,² E. G. HOOD,³ AND C. A. GIBSON⁴

Department of Agriculture, Ottawa, Canada

Cheddar cheese made from milk to which a small amount of commercial lipase has been added will develop a flavor defect known as rancidity (1, 2). This effect is presumably due to lipolysis of butterfat and the consequent liberation of fatty acids, the lower members of which possess an unpleasant odor (3). Rancidity produced in this manner closely resembles the odor of rancid cheese which occurs occasionally but persistently under factory conditions. It has been suggested that milk lipase may be among the factors concerned in the development of rancidity under commercial conditions (4). Since lipases from various sources would be expected to possess many properties in common, the study of lipase induced rancidity is of interest in connection with the rancid cheese problem.

It has been known for some time that lipase is protein in nature (5), and that as such it is subject to inactivation by proteolysis (6). These observations have been extended by their application to the study of cheese flavor defects. In this investigation experimental evidence is submitted to show that rennet or pepsin or both inactivate, to some extent, added lipase in cheddar cheese and that the use of higher amounts of these proteolytic enzymes contributes to flavor improvement of such cheese.

EXPERIMENTAL

Milk was obtained from the Experimental Farm herd. Small vats of cheese were made using 240 pounds of milk. This gave two 10–12 lb. cheese to each vat. Cheese were made by an experienced maker according to standard procedures except as otherwise indicated below. They were stored at 60° F. until the first grading and then transferred to a second storage held at 48–50° F. The cheese were graded at intervals for flavor only by an experienced member of the Dominion Grading Staff.

It was first necessary to determine the amount of commercial lipase (Pfanstiehl) which when added to cheese milk would develop rancidity in 10 to 14 days. This was done by making a series of vats of cheese using varying amounts of lipase. It was found that 2.5–5.0 gms. of lipase per 1000 pounds of milk gave a satisfactory working level.

It was possible to show in preliminary experiments that higher than the

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usual amount of Hansen's rennet (*e.g.* 6 oz. per 1000 lb. milk) retarded to a certain extent the development of rancid flavor by lipase in cheese. The same effect was demonstrated for pepsin and for mixtures of rennet and pepsin. Pepsin from two sources was used; Armour's pepsin made for cheese making during the last war, and Merck's soluble pepsin. These are referred to later as pepsin A and pepsin M, respectively. Papain and trypsin (both Eimer and Amend products) were also tried but, owing to a bitter licorice-like flavor of the former and a high lipase content of the latter their use was discontinued.

The process of making cheddar cheese is not a rigid procedure. Such factors as the daily variation in the composition of milk, the starter culture, the rate of acid development, and the details of the manufacturing process are difficult to control exactly under experimental conditions. These make the interpretation of the results of grading rather difficult. In order to eliminate these variables the following method was adopted. In the morning all milk was pooled, brought to temperature and the starter culture added. When nearly sufficient lactic acid had developed, the milk was divided into two equal portions. To each vat was added an equal amount of lipase suspended in water. One vat was coagulated with a low amount of rennet while the other received a higher amount than normal and the make was then completed in the usual manner. Pepsin alone, and pepsin and rennet mixtures were also used instead of rennet. In this way each vat was as nearly identical as possible in every respect except in the amount of proteolytic enzymes used. Accordingly, one vat served as a comparison or reference standard for the other. Variation in the flavor score could then be attributed mainly to the effect of proteolytic enzymes on added lipase in the cheese.

The results are summarized in the accompanying table. Comparison vats (or mutual reference standards) as explained above, are indicated by A and B following the same number. The cheese of series A have in each case a lower content of proteolytic or coagulating enzymes than the corresponding members of the parallel B series. The amounts of rennet, pepsin and lipase are shown in the second column. The succeeding columns show the successive flavor scores on the respective cheese at indicated intervals.

An examination of the results shows that in general cheese of the B series have a higher flavor score than their corresponding mates of Series A. Since the cheese of the B series have also a higher content of proteolytic enzymes it is concluded, therefore, that proteolytic enzymes enhance cheese flavor by preventing the onset of rancidity due to added lipase.

DISCUSSION

In order to assess the value of the above experimental data accurately, careful consideration must be given to the various factors involved.

It will be noted that there are a few exceptions to the general statement

TABLE 1
Rennet, pepsin, lipase and flavor of experimental cheese

Cheese	Treatment per 1000 lbs. of milk			Flavor scores ¹ of cheese during curing							
	Lipase	Rennet	Pepsin	Age in days	Score	Age in days	Score	Age in days	Score	Age in days	Score
	gm.	oz.	gm.								
1 A	5	2		9	38	16	38-	23	37-	86	37
1 B	5	3½		9	39-	16	38+	23	36	86	37
2 A	2.5	2		11	38	18	38	32	36-	60	39-
2 B	2.5	6		11	38+	18	39-	32	38-	60	39
3 A	2.5	2		10	38-	17	37	31	37-	59	36
3 B	2.5		86M	10	39-	17	38-	31	38-	59	36+
4 A	5	3½		11	38-	18	37	25	36	88	36
4 B	5	3	30A	11	39	18	37-	25	36+	88	37-
5 A	5	3½		10	38	17	38	24	36	45	36
5 B	5	3	43M	10	39-	17	38	24	38-	45	38
6 A	2.5	3		9	38	16	38+	30	36	58	37+
6 B	2.5	3	43M	9	39-	16	38	30	38	58	37

¹ Flavor standards for grades of Canadian cheddar cheese.

First grade cheese—minimum score for flavor 39

Second " " " " " " 37

Third " " " " " " < 37

unless below third grade.

that the flavor scores on cheese in series B are higher than those of corresponding cheese in Series A. No explanation is offered for this discrepancy. In any event these exceptions do not invalidate the general conclusion.

Also, admittedly, some of the differences between the flavors on corresponding cheese are small. However, because of the system of comparison vats more significance can be attached to these results than could otherwise have been possible. The variations between any two different gradings is subject to some uncertainty because of the variation in the judgment of an individual grader at two periods remote from each other. However, differences in this direction are of secondary importance in this investigation.

It must also be borne in mind that if, on the one hand, larger amounts of lipase than the above established level are used, rancidity in cheese is developed to a greater intensity than that encountered commercially. The lipolysis of cheese fat is extensive during the initial stage and the beneficial effect of proteolytic enzymes cannot undo the damage already in existence. On the other hand, when smaller amounts of lipase are used in order to decrease the rate of lipolysis so that the remedial effect of rennet or pepsin or both can be established early, the differences between the flavor scores on

any two corresponding cheese are reduced. The interpretation of results is thus made more difficult. In obtaining the above data it was therefore necessary to select an arbitrary working level of moderate sensitivity.

The differences in the flavor score between corresponding cheese of the series A and B are quite consistent at the period of their first grading. This observation is of some importance because cheese are generally graded at 10-14 days in commercial practice. Although the results of subsequent gradings have also been included and the same general trend is shown, the maintenance of a high score flavor over prolonged periods of storage can be considered as a problem separate from that of the initial flavor scores.

Mention might be made of the types of flavor obtained in the above experimental cheese. It is possible to obtain typical butyric rancidity particularly in those cheese containing higher than the adopted lipase level. With lower amounts of lipase the flavors were often described as not straight rancid, unclean, dirty, etc. It has been pointed out in our preliminary communication (4) that these flavors resemble those known to occur commercially. Therefore, it has been suggested that lipase may not only be responsible for the rancid flavor but to some extent for certain less defined flavor defects.

Assuming that naturally present milk lipase does play a part in the development of cheese flavor (7) the above results suggest an additional role for the proteolytic enzymes in cheese. In addition to its coagulating function with its contingent effect on texture, and the proteolysis of casein or chemical ripening, rennet may play an active part in the inactivation of lipase, which when present in abnormal amounts in cheese milk would be expected to produce undesirable flavors.

Lipase induced rancidity has not been definitely identified with the rancid flavor occurring under factory conditions. Other factors may also be involved. The results of this investigation, however, are submitted because of a possible existing relationship. At the same time additional information on the proteolytic function of rennet has been obtained. Further study of lipase and its possible relation to rancidity in commercial cheddar cheese is being continued.

SUMMARY

Rancid and other less defined flavors have been reproduced in cheddar cheese by the addition of commercial lipase to cheese milk.

A higher flavor score was obtained in cheese to which lipase was added when higher amounts of pepsin or rennet or both were used in its manufacture than in corresponding cheese where smaller amounts of proteolytic enzymes were added.

An additional function has been attributed to the rennet enzymes.

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UTILIZATION AND EXCRETION OF ASCORBIC ACID BY THE DAIRY COW*

C. A. KNIGHT, R. A. DUTCHER, N. B. GUERRANT

Department of Agricultural and Biological Chemistry

AND

S. I. BECHDEL

*Department of Dairy Husbandry, The Pennsylvania State College,
State College, Pennsylvania*

The results of early studies (1, 2, 3) concerning the influence of diet upon the antiscorbutic potency of cow's milk, appeared to indicate that the vitamin C content of the milk paralleled that of the ration. During the same period, however, findings were reported (4) which led to the opposite conclusion, namely, that the ration received by cows had no influence on the antiscorbutic potency of their milk.

Since the development of chemical methods for the quantitative determination of the antiscorbutic factor, which was shown to be ascorbic acid, further differences of opinion have arisen concerning the factors which influence the vitamin C content of cow's milk. As a result of recent work (5, 6, 7) the influence of breed and stage of lactation have been emphasized. Several workers (5, 8, 9) have attributed variations in the ascorbic acid content of milk to the season of the year. Other investigators (10, 11, 12, 13, 14) have concluded that the vitamin C content of cow's milk tends to be quite constant and is independent of the ration of the cow. Opposed to this view are those who still contend that the ascorbic acid content of the diet is a factor which cannot be ignored (6, 15).

A number of investigators have suggested that the cow is able to synthesize vitamin C (9, 13, 16, 17, 18, 19). How or where this synthesis occurs is not understood, although one investigator (30) has claimed that the vitamin C of cow's milk is synthesized by the udder parenchyma and that the synthesis depends largely upon the condition of the udder.

The failure of numerous experiments involving standard dairy rations to establish clearly and conclusively the importance of the ascorbic acid content of the diet with regard to the amount of the vitamin in the milk, made it seem desirable to repeat previous work, but to alter the procedure by supplementing standard rations with known amounts of pure synthetic ascorbic acid. Moreover, it was desired to increase the significance of customary milk ascorbic acid analyses by determining, simultaneously, the amount of ascorbic acid in the blood and in 24-hour samples of urine.

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If, in the above proposed studies, it could be shown conclusively that the vitamin C content of milk is independent of the ration of the cow, it was hoped that some explanation could be obtained for the anomalous results just mentioned. It was desired, for example, to find new evidence for the synthesis of ascorbic acid in the cow. Further, it was hoped that some clue might be found regarding both the metabolic fate of ascorbic acid and the factors which influence its elimination from the body in the milk and in the urine.

EXPERIMENTAL

Methods and Apparatus. One or both of two chemical methods of estimation were used in all ascorbic acid analyses. These were the Tillmans titration method (20) employing standard 2,6-dichlorobenzenoneindophenol, and the newer Roe furfural method (21), which estimates total ascorbic acid (both reduced and reversibly oxidized ascorbic acid).

Milk samples were taken in a special apparatus which has been shown to preserve all of the ascorbic acid of the milk in the reduced form (22). The use of this apparatus permitted the quantitative determination of the vitamin by the convenient indophenol titration method.

Blood samples were taken in the conventional oxalated tubes under paraffin oil. For each test, 20 to 30 ml. of blood were taken from the jugular vein of the cow. As soon as the sample was obtained, the collection apparatus was placed inside a dark glass receptacle containing ice and water and was taken immediately to the laboratory for analysis. Essentially the Farmer and Abt macro-method (23) for plasma ascorbic acid was used for routine blood analysis.

Successful collections of the total 24-hour urinary excretion, free from fecal contamination, were made by employing a special rubber urine tube developed by Forbes and coworkers (24). The 18-liter carboys, which were used as receivers in the collection apparatus, were painted black to exclude light and were charged with enough glacial acetic acid to give a final concentration of about 5 per cent by volume. Addition of stick metaphosphoric acid to the acetic acid appeared to give no better results than when acetic acid was used alone; consequently, the addition of metaphosphoric acid was discontinued. In urinary ascorbic acid analyses, both the indophenol titration and the Roe furfural method were employed, with the exception of the earliest work on one of the cows which was done before the furfural technique had been reported. This double analysis seemed desirable in view of the lack of unanimity among various workers concerning the specificity of present methods for the determination of ascorbic acid in urine. Moreover, it appeared probable that some of the vitamin would be unavoidably oxidized to dehydroascorbic acid during the collection of a 24-hour sample. Such has since been shown to be true with human urine (25). The furfural method

is particularly valuable in this case because it determines both dehydro- and reduced ascorbic acids.

It was recognized at the outset that it would be desirable to eliminate from the projected work the effect of breed differences by limiting the experiments to one breed of dairy cow. Holstein cows were eventually chosen, largely because they were available for experimental purposes at the time this work was started.

Effect of a Standard Ration. In order to have values which might later be used to compare with those obtained during administration of ascorbic acid, each cow was maintained on a standard ration for an interval of time during which analyses were made to determine the concentrations of ascorbic acid in the blood, the milk, and the urine. The averages of the values obtained during typical five-day test periods are given in table 1. Five-day

TABLE 1

*Summary of results obtained when Holstein cows were fed a standard ration and when they were fed a standard ration supplemented with ascorbic acid**

	S. R.	C. S.	G. C.	D.
Mg. ascorbic acid per ml. milk	0.019	0.021	0.021	0.020
Mg. ascorbic acid per 100 ml. plasma	0.53	0.58	0.50	0.48
Mg. ascorbic acid per day in urine—				
Indophenol titration	45.7	52.7	54.1	26.8
Furfural method	910.5	833.1	643.5	

S. R.—Standard ration.

C. S.—Standard ration supplemented with 50 to 100 grams of ascorbic acid mixed with corn silage.

G. C.—Standard ration supplemented with 50 grams of ascorbic acid in gelatin capsules.

D.—Standard ration supplemented with 50 grams of ascorbic acid administered by drenching.

* The values given in Table 1 represent the averages obtained in experiments employing from two to four cows, except in the case of the ascorbic acid administered by drenching, where the values are from an experiment with a single cow.

sampling periods were considered representative for any diet, in view of the fact that all cows received a particular ration for several days, or even weeks, prior to the actual collection of samples.

Effect of Ascorbic Acid Added to Corn Silage. In choosing methods for administration of massive amounts of ascorbic acid, it was decided to administer some of the vitamin mixed with a small amount (3–5 pounds) of corn silage. It was found that 50–200 grams (1,000,000–4,000,000 International Units) of ascorbic acid placed in such a mixture were consumed by a cow in a period of about 10 minutes. In contrast, ascorbic acid mixed with grain was eaten very slowly, if at all.

Experimental periods were designed to include a two-day interval during which the cow received the standard ration alone, followed by a three-day period during which she received the standard ration supplemented each day with a certain amount of crystalline ascorbic acid mixed with corn silage,

which was succeeded by a post-administrative period of two days during which the animal again received only the standard ration. The same procedure was used in subsequent experiments during which the ascorbic acid was administered in gelatin capsules and by drenching. The data from such experimental periods are summarized in table 1.

From a comparison of values in table 1, it is apparent that supplements of ascorbic acid administered by three different methods had no significant influence upon the concentrations of ascorbic acid in the blood, milk, or urine of the cows used in these experiments.

Effect of Ascorbic Acid Injected Intravenously. Rasmussen and coworkers (26) reported that intravenous injection of ascorbic acid resulted in a marked temporary rise in the vitamin C content of the milk of the ewe and cow. It was desired to repeat this work and to enlarge upon it by making analyses of blood and urine as well as of milk. Therefore, experiments were performed during which ascorbic acid was injected into the blood stream via the jugular vein. 24 grams of crystalline ascorbic acid, dissolved in sterile water, were given in this manner on each of two or three successive days. The effect of these ascorbic acid injections was studied with three cows with corresponding results. Typical values are given for one of the cows in table 2.

TABLE 2

Concentration of ascorbic acid in the blood, the milk, and in the urine of a Holstein cow during feeding of a standard ration supplemented with intravenous injections of ascorbic acid

Day	Mg. ascorbic acid per 100 ml. plasma	Mg. ascorbic acid per ml. milk	Mg. urinary ascorbic acid per day	
			Indophenol titration	Furfural method
1	0.58	0.021	24.3	611.1
2	0.58	0.020	34.6	241.5
3*	2.74**	0.020	6922.9	13311.8
		0.019		
		0.022		
4*	4.42**	0.024	9870.0	13632.6
		0.028		
		0.028		
5*	4.86**	0.030	14715.2	18194.0
		0.029		
		0.027		
6	0.86	0.028	8813.6	20923.8
		0.025		
		0.022		
7		0.021	161.2	604.7
8		0.020	117.8	982.8

* 24 grams of ascorbic acid dissolved in 100 ml. of sterile water were injected into the jugular vein. The multiple values for milk ascorbic acid represent each of the three daily milking periods.

** Blood analyses were made on samples taken 1½ hours after injection of ascorbic acid.

The intravenous injection of ascorbic acid produced unmistakable increases in the concentration of that vitamin in the milk and urine as well as in the blood. A very large portion of the injected ascorbic acid which could be accounted for appeared in the urine. In the case of one cow, this amounted to over ninety per cent of the total ascorbic acid injected. These results correspond closely to those recently reported (31) for experiments in which ascorbic acid was injected into goats.

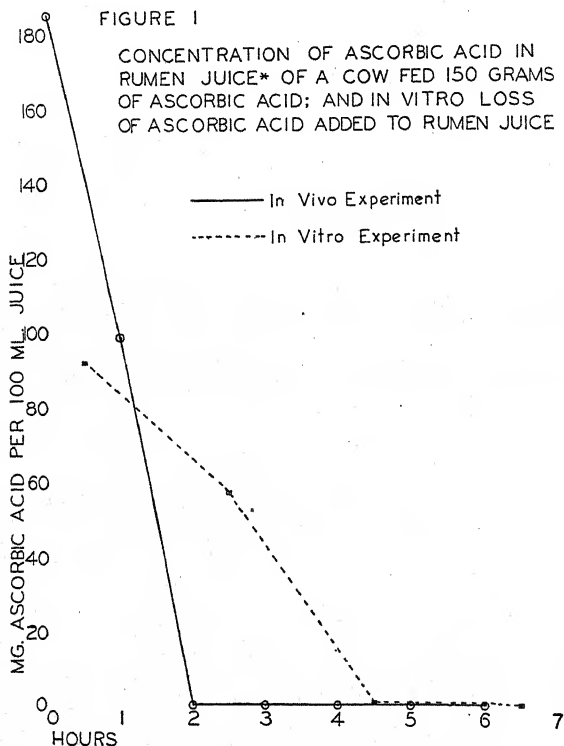
Effect of Ascorbic Acid Injected Subcutaneously. The experiments described thus far seemed to indicate that ascorbic acid administered by non-injection methods never reached the blood stream in significant amounts. Further, data from the intravenous injection experiments showed that the concentration of ascorbic acid in the milk was definitely increased if large amounts of the vitamin reached the blood stream. In order to further substantiate these findings, it was desired to administer some ascorbic acid by a method which would not place the vitamin directly in the blood stream but which would insure the ultimate arrival of large amounts in the circulation. Such a purpose was accomplished by setting up an experiment in which ascorbic acid was injected subcutaneously in regions around the cow's forelegs.

The effect of the subcutaneous injections of ascorbic acid closely resembled that of the intravenous injections. The influence of the subcutaneous injections upon blood, milk, and urinary ascorbic acid values was more gradual and somewhat less pronounced than in the case of the intravenous injections. During the course of three successive daily injections of 24 grams of ascorbic acid, the ascorbic acid titer of the milk was raised from 0.020 to 0.027 mg. per ml. and that of the urine from 600 to a peak of 15,000 mg. per day.

Studies with a Rumen Fistula. At this point in the experiments it appeared to be almost certain that the failure of massive amounts of ingested ascorbic acid to influence the concentration of the vitamin in the milk or to significantly alter its concentration in the blood and urine, could be attributed to a destruction of this substance in the rumen. To investigate this possibility, a rumen fistula was created in one of the cows. With this permanent opening leading directly into the largest compartment of the cow's stomach, it was possible to study the fate of ingested ascorbic acid by removal and analysis of partially digested food at intervals after feeding.

A rapid and pronounced destruction of ascorbic acid in the rumen was demonstrated by removal and analysis of samples of the rumen contents at regular intervals after feeding supplements of ascorbic acid and after insertion of the vitamin directly into the rumen. During such periods, the average concentrations of ascorbic acid in the blood plasma, in the milk, and in the urine were respectively 0.42 mg. per 100 ml., 0.019 mg. per ml., and 1260.5 mg. per day, values which do not differ significantly from those obtained during feeding of a standard ration alone.

The disappearance of ascorbic acid from the rumen contents during an experiment in which 150 grams of ascorbic acid were fed is shown graphically in figure 1. A preliminary report on this experiment has been given elsewhere (32).



* By the term "rumen juice" is meant the liquid portion of rumen contents.

In order to demonstrate that volume changes were not responsible for the pronounced decrease in the ascorbic acid concentration of the rumen contents as shown above, an experiment was performed in the laboratory with a controlled volume of rumen contents. 1000 ml. of rumen contents, from which the coarse particles of feed had been removed by straining through cheesecloth, were placed in a dark-glass, wide-mouth bottle. To this mixture, which had a pH of 6.50, was added 1000 mg. of crystalline ascorbic acid. After thoroughly mixing the contents, the bottle was loosely stoppered and placed in a water bath maintained at a temperature of 39° to 42° C. At intervals, samples were removed for analysis.

Figure 1 shows that the ascorbic acid disappeared just as it had in the *in vivo* experiments with the exception that the *in vitro* decrease proceeded at a more gradual rate. This slower rate of destruction of ascorbic acid in the *in vitro* experiment as compared to the *in vivo* experiments seems readily

explained by the absence of the continual stirring and the circulation of gases so characteristic of the rumen.

DISCUSSION OF RESULTS.

State of Ascorbic Acid in Urine. As soon as the furfural method was applied to the urine analyses it became apparent that the results obtained ranged from one and a half to thirty times as high as those obtained by the indophenol titration. During injections of ascorbic acid, however, this discrepancy narrowed to a point where, in some cases, the results obtained by the two methods almost coincided. These facts, together with the knowledge that the indophenol and furfural methods of analysis checked well when applied to 24-hour samples of rat urine collected under similar conditions (33), suggested that important amounts of the ascorbic acid excreted daily by a cow on a standard ration were excreted in the form of dehydroascorbic acid. In order to test this hypothesis, samples of urine were collected as they were excreted and analyzed at once. Table 3 gives the results obtained

TABLE 3

Reduced and oxidized ascorbic acid in cow's urine immediately following urination

Cow No.	Reduced ascorbic acid Mg. per liter Indophenol titration	Total ascorbic acid Mg. per liter Furfural method
1	20.9	17.0
2	33.2	25.4
3	19.7	18.2
4	10.1	10.3
5	27.7	25.1
6	27.6	25.4
7	53.1	48.1
8	40.1	43.3
9	21.5	19.0
10	31.0	29.6

from Holstein, Jersey, Brown Swiss, and Ayrshire cows. These findings seem to indicate that all the ascorbic acid excreted in the urine of the dairy cow is in the reduced form. The greater values obtained in eight out of ten cases by the indophenol method are readily explained by the recognized tendency to go beyond the endpoint, which is obscured to an extent proportional to the concentration of the urine pigments. The greater values obtained by the furfural method for 24-hour samples of urine must indicate, therefore, that variable amounts of the excreted ascorbic acid are oxidized to dehydroascorbic acid during the interval between excretion and analysis, or that the urine contains appreciable amounts of non-ascorbic acid furfural precursors capable of forming a derivative with 2,4-dinitrophenylhydrazine. Tests for the latter interfering substances were made repeatedly and in no case showed the presence of concentrations sufficiently large to interfere with

the method. Consequently, it may be concluded that while all the ascorbic acid excreted in the urine is originally in the reduced form, some of it is oxidized to dehydroascorbic acid under the conditions involved in the collection of a 24-hour sample of urine.

Destruction of Ascorbic Acid in the Rumen. Several explanations might be given for the rapid and pronounced disappearance of ascorbic acid from the rumen after oral administration of massive amounts of the vitamin. It might be argued that the large amounts observed in the first two or three samples represented stages in the incomplete mixing of the ascorbic acid with rumen contents. Changes in the concentration of the rumen ascorbic acid could also be attributed to an intake of water by the animal, or by a passage of a portion of the rumen contents to other chambers of the stomach.

Another interpretation of the findings is that the very soluble vitamin was rapidly absorbed. This has been suggested by Riddell and Whitnah (27). These workers studied the fate of vitamin C in the rumen contents of a cow with a rumen fistula and in a steer at slaughter. In each case the rumen contents were found to contain less than one-tenth the vitamin C of green rye ingested twelve hours earlier. These workers explained the rapid disappearance of ascorbic acid from the rumen by suggesting that ascorbic acid was rapidly absorbed. The basis for this suggestion was the observation of a temporary doubling of the vitamin C content of the blood within 12 hours and a fivefold increase in the ascorbic acid content of the urine within 60 hours after green feed was first supplied.

The most plausible explanation, however, and the one demanded by our experimental data, is that a rapid and pronounced destruction of ingested ascorbic acid occurs in the rumen. Thus, while the other factors which have been mentioned undoubtedly have some influence on the results observed, it is hardly likely that they account for changes of the magnitude indicated in figure 1.

The incomplete mixing theory is especially inadequate. When a cow is eating, the rumen contracts about three times per minute and each contraction causes a flow of liquid throughout the rumen and its solid contents. While figure 1 indicates that the first sample was removed at zero time, it was actually taken about five minutes after the animal had eaten the last of the ascorbic acid treated silage or 15 minutes after she had started to eat. This means that the ascorbic acid had been subjected to 15 to 45 contractions of the rumen. Further, when the cow was slaughtered, it was found that the rumen contained 38 liters of liquid or semi-liquid material. If 150 grams of ascorbic acid were thoroughly distributed throughout this liquid, there should be a concentration equivalent to about 400 mg. per 100 ml. of juice. Figure 1 indicates that the concentration of ascorbic acid in the juice, as shown by analysis of the first sample removed, was about 185 mg. per 100 ml. of juice. From these facts, it would appear that fairly complete mixing had occurred, accompanied by extensive destruction of the vitamin.

Observations of this and other dairy cows show that they drink water infrequently. It is difficult to say how much and how often liquid material passes permanently from the rumen into other compartments of the stomach. Ewing and Wright (28), working with steers, found that the average rate of passage of food residues through the rumen and reticulum was 61 hours. In any event, credence in the volume-change explanation for the disappearance of ascorbic acid from the rumen is seriously discounted by the results of *in vitro* constant-volume experiments such as shown in figure 1.

Indirect evidence that ingested ascorbic acid is largely destroyed in the rumen rather than being rapidly absorbed is given in the blood, milk, and urine ascorbic acid values, which, with the possible exception of the urine, show no response to the feeding of 50 grams or more of ascorbic acid. Why there is always a small amount of ascorbic acid in the rumen, as there appears to be, is difficult to explain. Possibly the release of this vitamin from solid feed fragments proceeds gradually and at a rate slightly higher than the rate of destruction. We hope to continue the study of the factors responsible for the destructive effects described.

In the light of the demonstrated destruction of ascorbic acid in the rumen, it becomes clear why various methods of oral administration of the vitamin failed to produce a response in the milk or other body fluids. If the ascorbic acid administered in gelatin capsules had been able to survive rumen conditions and reach other compartments of the stomach, it seems likely that other results might have been obtained, for the ascorbic acid content of the milk of non-ruminating animals, *e.g.*, guinea pigs (26) and humans (8, 29), has been shown to be influenced by diet. In work with the rumen fistula, however, it was found that gelatin capsules were dissolved and the ascorbic acid was released after 10-15 minutes in the rumen.

SUMMARY

1. Special equipment was employed which permitted the complete collection from dairy cows of 24-hour samples of urine free from any fecal contamination. It was found impractical, if not impossible, to preserve all the ascorbic acid in such urine samples in the reduced form.

2. By the simultaneous application of the indophenol titration and the furfural method of analysis to freshly excreted samples of urine, it was possible to show that ascorbic acid is excreted in cow's urine in the reduced form.

3. Analysis of over 50 samples of blood obtained from four Holstein cows showed that the ascorbic acid content of the plasma ranged from 0.43 to 0.62 mg. per 100 ml. when the cows received standard dairy rations.

4. Ascorbic acid was administered to Holstein cows (a) mixed with a small amount of corn silage, (b) in gelatin capsules, (c) in aqueous solution, (d) intravenous injection, and (e) by subcutaneous injection. Administration of as high as 100 grams (2,000,000 International Units) of ascorbic acid per day for three days by a non-injection method failed to increase the ascor-

bic acid concentration of the milk or blood and had only a slight effect on the concentration of the vitamin in the urine. It was only by the injection methods that a significant increase in the ascorbic acid concentrations of the blood, milk, and urine could be demonstrated. The greatest increase in milk ascorbic acid concentration during experiments in which 24 grams of ascorbic acid were injected intravenously on each of three successive days, was from 20 mg. per liter to 30 mg. per liter.

5. A rumen fistula was made in a Holstein cow. Experiments were performed in which this cow was fed as much as 150 grams (3,000,000 International Units) of synthetic ascorbic acid at one time; similar amounts were also placed directly in the rumen through the fistula opening. A rapid and pronounced destruction of ascorbic acid in the rumen was demonstrated by removal and analysis of samples of the rumen contents at regular intervals. Ascorbic acid added to rumen contents *in vitro* and stored in a dark-glass, stoppered receptacle at 39°–42° C. disappeared at much the same rate as that of the *in vivo* experiments. In making analyses, both the indophenol titration and the Roe furfural method were employed.

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STORAGE OF DAIRY BULL SPERMATOZOA*

ERIC W. SWANSON AND H. A. HERMAN

University of Missouri, Columbia, Mo.

An appreciation of the limitations and possibilities of storing dairy bull semen and retaining its fertility is necessary in order to define the practical limits of artificial insemination with shipped or stored semen. While various diluents have been proposed and different storage temperatures have been suggested, there still remain many unexplained factors concerning the successful storage of dairy bull semen. One difficulty in semen storage research has been the lack of an accurate criterion of fertility. Since the time of survival with vigorous motility of bull spermatozoa stored undiluted at 40° F. has been shown to be correlated with the fertility of bull semen (14), this property of the semen may be used in evaluating storage methods more accurately than simple determination of time of survival.

Refrigeration has been recognized for some time as the most effective method of preserving bull semen (15). Spermatozoa normally live only a short while at body temperature, and room temperature (70° F.) has likewise been found too high for successful semen storage (17). The highest temperature at which spermatozoa may be stored successfully has been given as 50° F. (4), and longer viability was secured with reduction of the storage temperature to 35° to 40° F. (4, 7, 8, 17). It has been emphasized that the change of temperature of the semen must be gradual or irreversible immotility will result from temperature shock (2, 6).

Many diluents of various composition have been used with bull semen, but their main use has been in increasing the volume (15, 4). The addition of nutrient substances to these diluents did not increase the survival of spermatozoa in them (1). The buffering action provided by some diluents has increased the motility of spermatozoa (8). A diluent using egg yolk plus a buffer has been reported as causing a marked increase in survival and fertility of stored bull semen (11).

The secretions of the accessory sex glands were shown to be harmful to survival of spermatozoa and were entirely unsuitable as diluting fluid for bull semen (5, 10, 11). With horse and boar semen the removal of the sperm fluid and concentration of the spermatozoa has resulted in greater survival in storage (16, 9). Experiments along this line with bull semen were not successful in obtaining greater survival in storage.

Early investigators excluded air from the semen by a layer of paraffin

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oil (15). Recent work has shown that such procedure is not necessary and that spermatozoa live longer with free access to oxygen (12, 13).

Although there are records of cows being settled with semen eight days old, the practical limits of storage of bull semen have been given as only 12 to 30 hours (3).

This paper presents the results of studies to find the most practical method of storing bull semen so as to preserve its fertility. Investigations were made as to the desirable temperature for storage, the benefits to be derived from using various diluents, the possibility of removing the accessory sex gland secretions to improve survival, and the actual effectiveness of stored semen as measured by its fertility when used in artificial insemination.

EXPERIMENTAL AND RESULTS

The semen used in these experiments was collected by use of the artificial vagina during the winter and spring of 1940 from ten bulls in the University of Missouri dairy herd.

Motility determinations were made at $250\times$ magnification of a drop of semen placed on a microscope slide in a stage incubator at 100° F. Motility was rated from 0 to 5. No motility was 0, and 5 represented the very best grade of motility. Weak, oscillatory motion with less than 40 to 50 per cent of the spermatozoa moving was rated 1. Progressive degrees of motility from 1 to 5 were rated 2, 3, and 4. Unless otherwise stated, all storage was made in an ordinary electric refrigerator operating at about 40° F. As soon after collection as possible, nearly always less than 30 minutes, the semen was put in small sterile glass vials, stoppered with freshly paraffined corks, wrapped in paper towelling, covered with rubber cots, and placed in a tray of water at 40° F. in the refrigerator. Cooling was thus made gradual and was accomplished within an hour after collection. A part of each sample was stored untreated at 40° F. as control for part of the same sample which was used in the experiment.

Effect of temperature on viability of semen. The storage temperature was observed to have a marked effect upon the rate of loss of motility. Thirty-seven samples representing ten different sires were stored at 70° to 75° F., while duplicate samples of the same semen were stored at 40° F. The averages of the motility ratings of these samples at various storage periods are presented in figure 1. At the end of six hours the semen stored at 70° F. showed slightly more vigorous motility than did that stored at 40° F. After 16 hours storage, motility in samples stored at 40° F. was much superior to that in samples stored at 70° to 75° F. Sixteen of the 37 samples stored at 70° to 75° F. were non-motile at 24 hours, and at 40 hours all except one were dead, while semen from the same samples stored at 40° F. still had good, vigorous motility.

A few samples of semen were stored at 31° to 32° F. Motility ratings of semen stored at this temperature fell more rapidly than motility in semen stored at 40° F., but the spermatozoa were still viable by the time motility rating in semen stored at 40° F. was reduced to 1. Thus, although motility was reduced, the time of survival was not greatly reduced.

Effect of diluting solutions on sperm viability. Samples of semen were divided to compare the effect upon viability in storage when diluted with

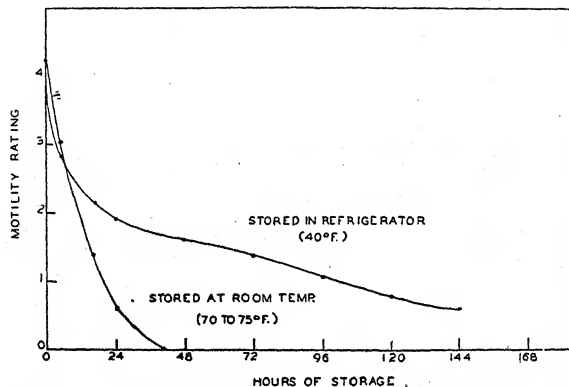


FIG. 1. Average maintenance of motility in 37 samples of semen stored at 40° F. and 70° to 75° F.

various diluents. The dilution rate used was three parts of diluent to 1 part of semen. The first diluents tried were 3 and 5 per cent glucose solutions and Milovanov's (10) S-G-C-2 dilutor. All of these were definitely harmful to the spermatozoa as measured by their effect upon motility maintenance. Motility was more vigorous in the undiluted semen in every case although the time of survival did not differ greatly between the diluted and undiluted semen.

The egg-yolk buffer (E-Y-B) dilutor which was proposed by Phillips *et al.* (11) was used in 18 samples of semen from eight different bulls. The comparison of the part of these samples diluted with E-Y-B dilutor with the part stored undiluted is shown in figure 2. On the average the use of this diluent was definitely favorable to sperm motility up to the fifth day of storage.

Thereafter, the semen which was diluted perished rapidly while that which was not diluted remained motile to a low degree. The effect of the E-Y-B dilutor varied greatly as to the semen of different bulls in which it was used. Semen from two bulls which was quite viscous and highly concentrated was greatly benefited by dilution with E-Y-B dilutor. Semen of these bulls was normally of very good initial motility, but the undiluted semen became very viscous in storage and motility declined rapidly. Semen of the other bulls which was more nearly normal in characteristics was

benefited very little if any by the use of this dilutor. In only one case, however, was the motility rating in the E-Y-B diluted semen less than that in the undiluted semen until the fifth day of storage. When the E-Y-B dilutor was added to undiluted semen which had become of low motility in storage, no reactivating or stimulating effect was secured even though a part of the same sample which had been stored diluted with E-Y-B dilutor initially had good motility at the time. In storage periods exceeding five days, the undiluted fractions of the semen survived longest.

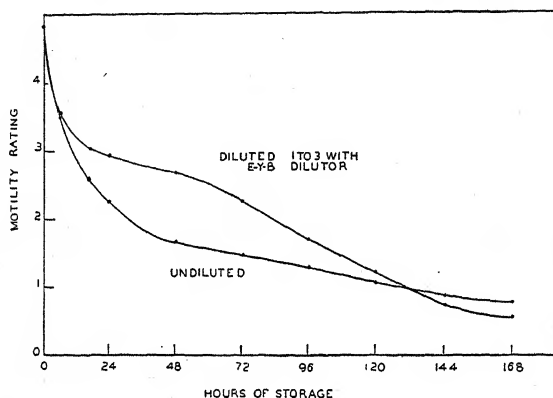


FIG. 2. Average motility ratings of semen stored undiluted and of semen from the same samples diluted 1 to 3 with egg-yolk buffer dilutor.

Effect of removal and replacement of the sperm fluid upon motility and survival of spermatozoa. Since the secretions of the accessory sex glands had been shown to be harmful to survival of the spermatozoa (5, 10, 11), the effect of removal of part of these secretions was studied. Separation was accomplished by centrifuging the fresh semen at 1300 RPM for 15 minutes. The relative centrifugal force was approximately 375 grams. The supernatant fluid which was clear and practically devoid of spermatozoa was removed by use of a pipette.

Spermatozoa stored in the concentrated state could not be reactivated with physiological saline or five per cent glucose solutions after 48 hours of storage, while the same semen untreated showed very vigorous motility at 48 hours. Replacing the sperm fluid with S-G-C-2 dilutor or three per cent glucose solution immediately after centrifuging proved of little value for maintaining motility. Although a low motility rating was maintained for three to five days in semen so treated, the motility in the untreated semen was very much better and was maintained long after the treated semen had died.

Replacing the sperm fluid with four volumes of E-Y-B dilutor immediately after centrifuging was decidedly beneficial to the spermatozoa for the

first four days of storage. The average results of such treatment of 14 samples of semen from six bulls compared with motility ratings of the same samples untreated are presented in figure 3. Here again it was noticed that very good motility was secured from spermatozoa stored in E-Y-B dilutor for four days. On the fifth day of storage, however, motility was as good in the untreated as in the treated semen; and thereafter the treated semen died rapidly while a low motility rating was maintained for some time in the natural semen. In six of the 14 samples, however, survival time was as long or longer in the treated as in the natural semen.

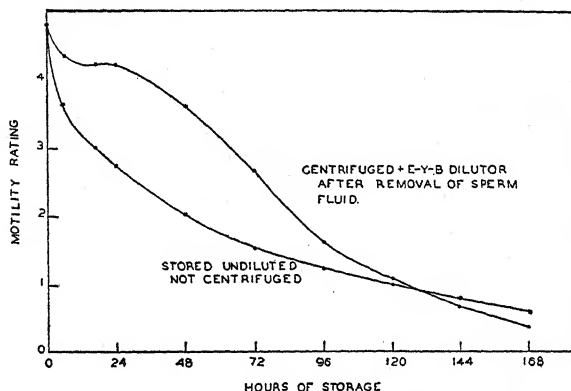


FIG. 3. Comparison of motility maintenance in semen stored undiluted and semen stored after replacing sperm fluid with egg-yolk buffer dilutor.

Since egg yolk contains practically no glucose, and since the sperm fluid containing glucose had been removed, glucose was added to three centrifuged E-Y-B diluted samples of semen with hopes of prolonging the life of the centrifuged spermatozoa. Semen so treated reacted practically the same as identical semen treated in the same way but without glucose. Hence glucose as such apparently was not the limiting factor in causing the sudden, early death of the centrifuged spermatozoa.

Insemination with stored semen. The stored semen was used for inseminations at every opportunity as it seemed that this practice should give the best test of maintenance of fertility. During the winter and spring of 1940, thirty inseminations were made with stored semen of which 15 resulted in conception. The complete record of these inseminations, including the age of the semen when used and its motility characteristics is given in table 1. These results show that the failure of certain samples of semen to produce conception was correlated with quality of the semen, irrespective of age. There was considerable variation even in samples from the same bull as to the time they could be stored successfully. The record indicates that the undiluted semen was as effective as either that diluted with E-Y-B dilutor or that centrifuged and diluted with E-Y-B dilutor. There is also indication

TABLE 1
Record of inseminations using stored semen

Bull No.	Age of semen when used (hrs.)	Motility rating at time of insemination	Maintenance of 2 motility in semen (hrs.)	Maintenance of 2 motility after use (hrs.)	Remarks
Inseminations which resulted in conception					
47	74	3	96	22	E-Y-B diluted
47	198	2	198	0	E-Y-B diluted; shipped 400 mi.
48	4	3	Shipped 118 mi.
48	68	1	24	0	Double insemination
48	11	3	24+	13+	Shipped 150 mi.
48	10	3	Shipped 150 mi.
49	48	5	72	24	Shipped 40 mi.
49	72	3	96	24	
54	44	4	144	100	
54	24	4	72	48	
40	115	3	120	5	
50	24	2	24	0	
50	4	4	24	20	Shipped 40 mi.; cow with vaginitis
50	72	2	120	48	
50	20	3	48	28	Double insemination-shy breeder
Ave.	52.5	3.0	81.6	25.5	
Insemination which failed to impregnate					
47	20	4	E-Y-B diluted, shipped 400 mi.
47	125	2	196	71	E-Y-B diluted
55*	55	1	24	0	E-Y-B diluted
55*	48	4	48	0	E-Y-B diluted
49	24	4	96	72	Cow was shy breeder
49	72	4	96	24	Same sample as above
49	120	3	144	24	
49	56	4	48	0	E-Y-B diluted and centrifuged
49	16	4	72	56	E-Y-B diluted and centrifuged
53*	20	1	6	0	Double insemination
53*	24	1	6	0	
50	96	2	96	0	
50	4	4	24	20	Heifer with vaginitis
50	4	4	24	20	Semen shipped 40 mi.
50	24	2	24	0	
57	5	1	0	0	Semen shipped 150 mi.; bull's first service in 2 weeks.
Ave.	47.2	2.7	62.9	19.1	

* Bulls of low fertility.

that semen of poor motility at the time of insemination was ineffective, although there were exceptions. One cow was settled with semen showing a motility rating of only 1. Some samples of semen were used on two cows, one of which conceived and the other did not, so evidently the genital mechanism of the cow is quite important in affecting the use of stored semen. The

oldest semen which resulted in conception (also the oldest tried) was stored 198 hours. A live calf has now been dropped as a result of this insemination. The next oldest semen was stored 115 hours. Three samples stored over 90 hours failed to produce conception. The ratio of two services per conception for use of all the stored semen, considering all types of cows and bulls included in the study, is not excessive. There is indication that a fair conception rate should result from use of good quality semen from fertile bulls which has been stored 48 to 72 hours, provided that it shows good motility at the time of insemination.

DISCUSSION

In handling or preparing semen for storage, much care is necessary. The full initial power and energy of the spermatozoa must be preserved insofar as possible. Temperature shock should be avoided by changing the temperature gradually. The storage temperature should be reached promptly after collection, however, and it should be low. Ordinary household refrigerator temperature of 40° F. has been found satisfactory. This study has clearly shown that room temperature (70° F.) is too high for storage of bull semen. Temperature of melting ice (32° F.) was too low for best motility maintenance, although semen can be kept alive for long periods at that temperature. It may be that the cooling and warming around 32° F. are too rapid, or that temperatures so low may have a deleterious effect on the spermatozoa.

The use of diluents to aid in preserving fertility of spermatozoa was not necessary. Although the egg-yolk buffer dilutor increased the vigor of motility for the first few days of storage, it did not prolong the life of good quality semen; and in the few actual inseminations of cows in which it was tried, it was not superior to the undiluted semen in fertility. In view of this fact, the main use of diluents seems to be still one of increasing volume. In cases where an increase in volume is desired, however, the egg-yolk buffer dilutor should be used in preference to others herein reported as it was the only one which gave a favorable effect to spermatozoa motility. The short beneficial action of the egg-yolk buffer does not seem to be by way of nutrition of the spermatozoa for they died quicker in it than they did in good quality undiluted semen stored at 40° F.

Increasing the concentration of bull spermatozoa was not conducive to sperm survival. Removal of the accessory sex gland fluids by centrifuging did increase motility, however, when a favorable diluent was used for replacement. Since storage of the concentrated spermatozoa was not successful, it appears that bull spermatozoa require some medium, probably for the elimination of waste products, for best survival. The special treatment necessary to centrifuge and dilute bull semen may have valuable application

in the shipment of semen over long distances where it will be used within 48 to 72 hours. Further practical work is necessary to prove this.

CONCLUSIONS

1. Ordinary household refrigerator temperature of 40° F. was entirely satisfactory for storage of bull semen.
2. Room temperature or near freezing temperature was undesirable for storage of bull spermatozoa, although the effect of the latter was not markedly injurious.
3. Glucose solutions and Milovanov's S-G-C-2 dilutor were of no value in increasing the length of survival time in stored bull semen.
4. Egg-yolk buffer dilutor (Phillips, 11) was beneficial to motility of bull spermatozoa for the first 100 hours of storage. Semen varied in its reaction to dilution with this diluent.
5. Very vigorous motility was obtained for the first 100 hours of storage by removing the sperm fluid from the spermatozoa and diluting with egg-yolk buffer dilutor.
6. Good quality semen survived longer when stored *undiluted* than when diluted with any of the diluents tried.
7. Good quality semen which has been stored undiluted at 40° F. can be used for insemination with a reasonable degree of fertility for storage periods up to two to three days. Conception with dairy bull semen stored at longer intervals is possible, as demonstrated in this study, but the practicability of using such semen is questionable in view of the low ratio of pregnancies resulting.

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QUANTITATIVE DETERMINATION OF ALPHA AND BETA LACTOSE IN DRIED MILK AND DRIED WHEY

PAUL F. SHARP AND HUGO DOOB, JR.

Cornell University, Ithaca, New York

INTRODUCTION

The physical properties of many milk products, particularly dried milk and whey, are greatly influenced by lactose. Therefore, it is important to know whether the lactose is in the crystalline state and, if so, whether crystallized as the alpha or the beta form and the extent to which crystallization has progressed.

The presence of alpha hydrate and beta crystals can be demonstrated qualitatively by using the seeding tests described by Sharp (2). Troy and Sharp (3) described briefly a method for determining the relative amounts of alpha and beta lactose. They did not give details because the method had not been studied sufficiently at that time. The present article gives the details of procedure and some of the supporting evidence on the points of technique involved. The basis of this method is to obtain quickly a clarified solution of the product, polarize the solution at once, allow the solution to stand until the forms of lactose reach equilibrium, and finally polarize again. The relative amounts of alpha and beta lactose are calculated from the change in rotation; the amount of total anhydrous lactose is calculated from the final rotation.

REAGENTS AND APPARATUS

Alcoholic mercuric chloride. Dissolve 264 grams of mercuric chloride in 1000 ml. of 95 per cent ethyl alcohol, hold overnight and filter. Each determination requires 10 ml.

Norrit suspension. Suspend 120 grams of norrit in 900 ml. of water, add enough N HCl to give a pH of 4-5 and then make up to 1 liter. Each determination requires 5 ml. of the freshly agitated suspension.

Previous to preparing the suspension 125 grams of norrit should be refluxed 10 hours with 1 liter of 1 per cent hydrochloric acid and washed by refluxing repeatedly with distilled water. Acid extraction is not necessary with some samples of norrit; also the alkalinity of some samples is not sufficient to require adjusting of the pH to 4-5 if heavily buffered solutions are analyzed.

Citric acid solution. A solution containing 0.2 to 0.3 per cent citric acid mono-hydrate is prepared. This solution is needed to obtain clear filtrates from badly heated or brown whey; otherwise water is used. Each analysis of dried whey requires 45 ml.

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Oxalic acid dihydrate. Crystalline solid, about 0.02 to 0.04 gram used for each determination; required for all dried milk.

Oxalic acid solution. A solution containing 0.9 gm. oxalic acid dihydrate per liter is prepared. All dried milk samples are dissolved in 45 ml. of this solution.

Zinc chloride. Crystalline solid about 0.1–0.2 gram used for each determination on dried whey if required.

Apparatus. Balance, porcelain mortar and pestle, 100 ml. volumetric flask, erlenmeyer flask, funnel, watch glasses, fluted crepe filter paper, polariscope, stop-watch, 4 decimeter water-jacketed polariscope tube and intense light (589 mμ).

METHOD

Clarification procedure. Weigh 2.500 grams of the dried milk or whey and transfer to a dry porcelain mortar (the sample taken should contain not less than 1 gram nor more than 2.5 grams of lactose). Dry grind the sample for approximately a minute in the mortar, add 2.0 ml. of distilled water at 17° C. to a whey, or 10 ml. oxalic acid solution at 17° C. to a milk. Start a stop watch and wet grind to produce a smooth thin paste which then grind vigorously. Add more solvent and continue to grind until about 30 ml. have been added. Pour the mixture into a 100 ml. volumetric flask, into which has previously been introduced 5 ml. of norrit suspension. Rinse the mortar with additional 3 to 4 ml. portions of solvent until a total amount, 45 ml. at 17° C., has been used. (If the final filtrate obtained from a dried whey is not clear the citric acid solution should be substituted for water up to this point.) Complete the rinsing with 15–25 ml. of water at 25° C. (wash bottle) and thus transfer the entire sample with the lactose completely dissolved and extracted, to the volumetric flask. Swirl the contents. Swirling is advised to reduce foaming.

Add to the volumetric flask 10 ml. of the ethyl alcoholic solution of mercuric chloride. Rotate and swirl the contents of the flask 0.5–1.0 minute in order to mix thoroughly. Make up to the 100 ml. mark with distilled water at 25° C. When alcohol and water are mixed a slight rise in temperature results. For this reason, the temperature of the water used in extracting and making up the solution should be adjusted so that the final temperature after mixing and diluting to the mark will be approximately 25° C. The elapsed time at this stage should be 2 to 3 minutes. Shake and mix the contents of the volumetric flask for 30 seconds and pour the entire contents of the flask at once onto a fluted crepe filter paper. (This filter should not be folded too tightly or broken paper fibers will be delivered with the filtrate.) Return the filtrate to the filter until delivered clear. Then filter directly into a dry, water-jacketed polariscope tube maintained at a temperature of 25° C. Collect the remainder of the filtrate in a dry erlenmeyer flask. Keep the filter covered to minimize evaporation.

Polariscopic readings. As soon as the polariscope tube is filled a reading is taken, and additional readings are taken at minute intervals until a total of 11 has been made. Unless the temperature of the solution is 25° C. at the start the first few readings will be in error. The first reading of the polariscope is usually obtained within 5 minutes after starting the stop watch. Occasionally the time will be somewhat longer, if the solution filters slowly.

The 11 polariscopic readings are plotted against the time, as indicated by the readings of the stop watch. The best line is drawn through these points, and it is extrapolated to zero time—that is, the time of adding the water and starting of the stop watch. The readings of the polariscope extrapolated to zero time give the initial rotation, *I*, of the solution. The final rotation is obtained after the solution has stood at least 8 hours. It is often most convenient to add a drop of toluene and allow the solutions to stand overnight at room temperature (25° C.), in flasks. Ten readings of the final rotation are made, and the average, called *F*, is used in the equations given below. If a precipitate forms on standing overnight, filter the solution. Whey filtrates in which colloidal material is suspended can usually be cleared by addition of 0.1–0.2 gram of solid zinc chloride. It may be necessary to heat the solution in a water bath for a few minutes until flocculation occurs. The solution is filtered through a fine-grained filter paper.

Milk filtrates generally become turbid and cannot be clarified satisfactorily by means of zinc chloride. These are treated with 0.02–0.04 gram of solid oxalic acid dihydrate and filtered through a fine-grained filter after precipitation is complete.

Calculations. The relative amounts of anhydrous alpha lactose or of beta lactose are obtained by substituting the initial (*I*) and the final (*F*) rotations in one of the two equations (7) or (8), given below:

$$\% \text{ anhydrous alpha lactose} = \left(\frac{I}{F} - 0.635 \right) 101.1 \quad (7)$$

$$\% \text{ beta lactose} = \left(1.624 - \frac{I}{F} \right) 101.1 \quad (8)$$

If information is required only as to the relative amounts of alpha and beta lactose present, it is not necessary to weigh the sample taken for the determination; but by taking an accurately weighed sample of 2.500 grams, the amount of lactose in the product can easily be determined from the final reading by multiplying the final reading by 18.15 if a 4 decimeter tube is used. If a saccharimeter instead of a polarimeter is used, the value must be changed to its equivalent reading on the saccharimeter scale.

SIGNIFICANCE OF DETAILS

The principle on which this method is based is simple and obvious. The difficulty arises in obtaining quickly a water-clear extract in which the

mutarotation of the sugar has not been accelerated by the protein precipitants. Most agents used for clarifying protein solutions are not satisfactory because they accelerate mutarotation, and we are thus limited to those precipitations which can be carried out in a neutral (pH 4-6) solution. A great number of protein precipitates were investigated both singly and in combination. An alcoholic solution of mercuric chloride was found to be the most satisfactory.

Solution of the sample. If the lactose is not dissolved before adding the alcohol, the rate of solution is generally so retarded by the alcohol as to delay the determination of the initial rotation; or some of the lactose may actually be filtered out if sufficient time is not allowed for solution. Relatively large lactose crystals are present in some samples, and solution is not sufficiently rapid unless the sample is dry ground in the mortar before the water is added.

Adding the water to the sample in the mortar permits the immediate disintegration of the lumps which form in many samples and enables the extraction to be made quickly and completely.

The extraction in the mortar is made at about 17° C. so that the heat liberated when the alcoholic mercuric chloride is added later will bring the temperature to 25° C. Unless the temperature is 25° C. at the time of the first polariscopic reading, the first few values will be in error; for the differences in refractive index produced by the difference in temperature of portions of liquid in the polariscope tube will make matching of the fields difficult.

The amount of water used at the various stages has been carefully worked out to permit the complete solution of the lactose and transfer of the sample to the 100 ml. volumetric flask, and to obtain a temperature of 25° C. in the final mixture.

Alcoholic mercuric chloride precipitation. Alcohol was used because it is a good solvent for mercuric chloride and because it also aids in the protein precipitation and prevents adsorption of lactose by the norrit. Norrit was required to remove the yellow color produced by the riboflavin and the brown color of the milk and whey produced by heat or aging. The amount of norrit recommended sufficed to decolorize all but abnormally brown (overheated or very old) samples. The slight residual yellow color of some filtrates obtained from such samples did not seriously reduce visibility in the polariscope.

Turbidity in a filtrate, slight enough to be almost imperceptible when viewed in a flask, becomes disturbing when viewed through a 4 dm. polariscope tube. A light of high intensity is recommended for fine work. We used a monochromator adjusted with calibrated quartz plates.

Varying concentrations of mercuric chloride in the alcohol, varying amounts of alcohol and varying amounts of norrit were tried and a combina-

tion of the three was worked out which gave the best results. If too much alcohol is used the solubility of the lactose is affected and also its specific rotation; yet sufficient alcohol should be used to aid in the precipitation and prevent adsorption of lactose by the norrit.

Norrit. The norrit is added in the form of a water suspension because the air contained in the dry material causes marked foaming, whereas in the suspension the air has been eliminated from the surface of the norrit.

A trace of lactose seems to adsorb on the norrit even in the presence of alcohol. Irregular behavior was occasionally observed when unextracted norrit was used. No correction is applied for the volume of the norrit (0.4 ml.) or of the protein precipitate. This is because the corrections are small, and because the amount of lactose adsorbed and the effect of the alcoholic mercuric chloride on the rotation of the lactose approximately compensate for the concentrating effect of the volume displaced by the solid phase.

To retard mutarotation, it is advisable to adjust the acidity of the norrit suspension to pH 4-5 with hydrochloric acid, and such adjustment is necessary when pure unbuffered lactose solutions are analyzed. In the analysis of dried milk and dried whey, if the highest accuracy is not desired, the extraction with hydrochloric acid and the adjustment of the pH of the norrit suspension can be dispensed with. The unextracted norrit suspensions tested had pH values of 8 to 9. The buffer value and pH of the dried milk are sufficient to reduce the pH and prevent marked acceleration of mutarotation by the alkaline norrit.

Clouding of filtrates and development of cloudiness: Most dried milks and some dried wheys, if dissolved in water, give turbid filtrates after precipitation with alcoholic mercuric chloride. A number of substances were added in the attempt to obtain clear filtrates and at the same time not interfere with the determination.

The best results were obtained with dried milks by dissolving them in the oxalic acid solution recommended. This served to delay the appearance of turbidity. Its mode of action is not clear. If the turbidity is due to a colloidal calcium compound, the oxalic acid might either delay its formation, or partially precipitate it.

Some dark samples of dried whey must be extracted with the citric acid solution as a solvent in place of water in order to obtain clear filtrates for determining the initial rotation.

Precipitation occurs in many whey filtrates and most milk filtrates during equilibration.

The turbidity of whey filtrates is usually removed by filtering through a fine paper; milk filtrates generally remain turbid. Turbidity in whey filtrates which is not removed by simple filtration can be precipitated by treatment with solid zinc chloride (0.1 - 0.2 gm.); in milk filtrates by treat-

ment with solid oxalic acid dihydrate (0.04 gm.). Warming accelerates flocculation. The flocculated material is removed with a fine filter.

Adding alkalis to accelerate final rotation. Many investigators recommend the addition of alkalis so that the final rotation can be obtained almost immediately after obtaining the initial rotation. Sodium carbonate, sodium bicarbonate and ammonium hydroxide have been recommended. This procedure, unless tested with the specific product, may lead to errors. If too much alkali is added the optical rotation of the sugar is affected. If not enough alkali is added, the mutarotation may not be accelerated as expected. Correct final rotations are obtained by acceleration with alkali only when the solution is adjusted to the proper pH. The addition of the same amount of alkali to different samples will not give the same pH because of the different buffer values, particularly in the case of dried wheys. Moreover, the addition of alkali precipitates mercuric oxide in the filtrates here employed. For these reasons it is much safer to allow the solution to reach equilibrium by standing rather than to attempt to accelerate mutarotation by alkali.

Derivation of equations. Several values are given in the literature for the specific rotations of the two forms of lactose and of their equilibrium mixture for the sodium D lines at 20° C. (1). Direct determinations at 25° C. are not reported. The specific rotations obtained in this laboratory at 25° C. with light of 589 mμ using products of high purity are as follows: anhydrous alpha lactose (weighed as the hydrate), + 89.5° beta lactose, + 35.0°; equilibrium mixture at concentrations between 10 and 250 gm. anhydrous lactose per liter, + 55.1°. All three constants apply to aqueous solutions.

Suppose A_0 per cent of the lactose in a sample were in the alpha form, then $100 - A_0$ represents the per cent in the beta form. The initial specific rotation, I_s , of a solution obtained from this sample would be given by

$$89.5A_0 + 35(100 - A_0) = 100 I_s \quad (1)$$

where the constants are the specific rotations of alpha and beta lactose respectively.

On equilibration at 25° C. the solution would assume a final specific rotation, F_s , and in this solution A_∞ per cent of the lactose would be in the alpha form, $100 - A_\infty$ per cent in the beta form. For this solution

$$89.5A_\infty + 35(100 - A_\infty) = 100 F_s \quad (2)$$

Dividing (1) by (2) and collecting terms gives,

$$\frac{I_s}{F_s} = \frac{54.5A_0 + 3500}{54.5A_\infty + 3500} \quad (3)$$

In the equilibrium solution at 25° C., however, we have:

$$89.5A_\infty + 35(100 - A_\infty) = 100 \times 55.1 \quad (4)$$

where 55.1 is the specific rotation of this solution. Solving (4) for A_∞ we obtain 36.9 and substituting this value in (3) and solving for A_0 :

$$A_o = \left(\frac{I_s}{F_s} - 0.635 \right) 101.1 = \% \text{ alpha} \quad (5)$$

and

$$(1 - A_o) = \left(1.624 - \frac{I_s}{F_s} \right) 101.1 = \% \text{ beta} \quad (6)$$

Since the observed initial and final rotations, I and F , are made with the same solution in the same polariscope tube they are proportional to I_s and F_s and can be substituted in (5) and (6) to give (7) and (8).

$$A_o = \left(\frac{I}{F} - 0.635 \right) 101.1 = \% \text{ alpha} \quad (7)$$

$$(1 - A_o) = \left(1.624 - \frac{I}{F} \right) 101.1 = \% \text{ beta} \quad (8)$$

These percentages, of course, refer to total amount of anhydrous lactose in the sample as 100.

Accuracy of the method. Tables 1 and 2 show results obtained when the method was applied to the pure alpha and beta forms of lactose respectively. Inspection of table 1 shows that filtration and such possible impurities in the alcohol as acetaldehyde and acetic acid do not influence the results. Mercuric chloride and alcohol, individually and combined, so affect the rotation as to give apparent percentages of total lactose slightly below 100, and apparent percentages of alpha lactose slightly above 100.

Norrit adsorbs appreciable amounts of lactose from aqueous solution but only a very limited amount from solutions containing alcohol.

The effects of norrit, alcohol, mercuric chloride and combinations of these reagents on beta lactose solutions are shown in table 2. The results are similar to those obtained with solutions of alpha lactose. In the presence of alcoholic Hg Cl_2 the apparent percentage of total lactose is a little low, but the percentage of beta lactose is as nearly correct as the errors in the method would justify. Adsorption of beta lactose on the carbon is shown and the adsorption is also inhibited by alcohol.

Preferential adsorption of alpha lactose on norrit. Not only does norrit adsorb appreciable amounts of lactose from an aqueous solution but it adsorbs the alpha modification preferentially. This can be demonstrated clearly when appreciable amounts of both the alpha and beta forms are present in the solution, and when enough norrit is added to adsorb about one half of the lactose present. A dry mixture containing 40 per cent by weight of alpha lactose (calculated on the anhydrous basis) and 60 per cent of beta lactose was prepared. Aliquots containing 0.74 gram anhydrous lactose were dissolved and made up to a final volume of 100 ml., using variations in procedure which would demonstrate adsorption of lactose by norrit and elution by alcohol. The results are presented in table 3.

Experiments A, B, C and D show no appreciable effect of filtration and only a slight effect of 20 per cent alcohol on the ratio and rotations of the

TABLE 1
Analysis of alpha lactose hydrate, sugar weighed on anhydrous basis

	Rotations 4 dm. tube, 25° C.			Amount of lactose		Total lactose in product %
	Initial I	Final F	$\frac{I}{F}$	As Alpha %	As Beta %	
Water and lactose alone	8.96	5.511	1.626	100.2	-0.2	100.0
	8.96	5.508	1.627	100.3	-0.3	100.0
	8.93	5.504	1.622	99.8	0.2	99.9
	8.94	5.508	1.623	99.9	0.1	100.0
Water and lactose alone, filtered	8.96	5.488	1.633	100.9	-0.9	99.6
	8.93	5.482	1.629	100.5	-0.5	99.5
	8.95	5.510	1.624	100.0	0.0	100.0
	8.92	5.514	1.618	99.4	0.6	100.1
In 4.5% alcohol	8.95	5.492	1.630	100.6	-0.6	99.7
In 9 % alcohol	8.94	5.479	1.632	100.8	-0.8	99.4
In 18 % alcohol	8.87	5.449	1.628	100.9	-0.9	98.9
In 4.5% alcohol, filtered	8.96	5.489	1.632	100.8	-0.8	99.6
In 9 % alcohol, filtered	8.94	5.479	1.632	100.8	-0.8	99.4
In 18 % alcohol, filtered	8.81	5.437	1.620	99.6	0.4	99.4
In 1.2% HgCl ₂	8.98	5.480	1.639	101.5	-1.5	99.5
In 2.3% HgCl ₂	8.94	5.472	1.634	101.0	-1.0	99.3
In 4.5% HgCl ₂	8.94	5.474	1.633	100.9	-0.9	99.4
In 1.2% HgCl ₂ , filtered	8.98	5.464	1.643	101.9	-1.9	99.2
In 2.3% HgCl ₂ , filtered	8.97	5.475	1.638	101.4	-1.4	99.4
In 4.5% HgCl ₂ , filtered	8.91	5.466	1.630	100.6	-0.6	99.2

TABLE 1—(Continued)

	Rotations 4 dm. tube, 25° C.			Amount of lactose		Total lactose in product %
	Initial I	Final F	$\frac{I}{F}$	As Alpha %	As Beta %	
+ 10 ml. 26.4% alcoholic HgCl ₂	8.85	5.457	1.622	99.8	0.2	99.0
	8.88	5.445	1.631	100.7	-0.7	98.8
+ 10 ml. 26.4% alcoholic HgCl ₂ , filtered	8.90	5.473	1.626	100.2	-0.2	99.3
	8.91	5.484	1.625	100.1	-0.1	99.5
	8.92	5.479	1.628	100.4	-0.4	99.4
+ 20 ml. 26.4% alcoholic HgCl ₂ , filtered	8.85	5.431	1.630	100.6	-0.6	98.6
+ 10 ml. 26.4% alcoholic HgCl ₂ containing .75% acetaldehyde	8.92	5.466	1.632	100.8	-0.8	99.2
+ 10 ml. 26.4% alcoholic HgCl ₂ containing .25% acetic acid	8.95	5.467	1.637	101.3	-1.3	99.2
+ .60 gm. norrit in suspension	8.52	5.236	1.627	100.3	-0.3	95.0
	8.56	5.257	1.628	100.4	-0.4	95.4
+ 10 ml. 26.4% alcoholic HgCl ₂ + .60 gm. norrit in suspension	8.85	5.450	1.624	100.0	0.0	98.9
	8.85	5.449	1.624	100.0	0.0	98.9
	8.82	5.451	1.618	99.4	0.6	98.9
	8.85	5.451	1.624	100.0	0.0	98.9

TABLE 2
Analysis of beta lactose

	Rotations 4 dm. tube, 25° C.			Amount of lactose		Total lactose in product %
	Initial I	Final F	$\frac{I}{F}$	As Alpha %	As Beta %	
Water and lactose alone	3.51	5.514	.637	0.2	99.8	100.1
	3.48	5.492	.634	-0.1	100.1	99.7
Water and lactose alone, filtered	3.50	5.529	.633	-0.2	100.2	100.4
	3.52	5.527	.637	0.2	99.8	100.3
+ 10 ml. 26.4% alcoholic HgCl ₂	3.49	5.489	.636	0.1	99.9	99.6
	3.46	5.476	.632	-0.3	100.3	99.4
+ 10 ml. 26.4% alcoholic HgCl ₂ filtered	3.47	5.476	.634	-0.1	100.1	99.4
	3.48	5.475	.636	0.1	99.9	99.4
+ .60 gm. norrit in suspension	3.36	5.249	.640	0.5	99.5	95.3
	3.33	5.257	.633	-0.2	100.2	95.4
+ .60 gm. norrit in suspension	3.54	5.467	.648	1.3	98.7	99.2
	3.51	5.485	.640	0.5	99.5	99.6
+ 10 ml. 26.4% alcoholic HgCl ₂	3.47	5.494	.632	-0.3	100.3	99.7
	3.47	5.478	.633	-0.2	100.2	99.4

forms of lactose. In experiment E about half of the lactose was adsorbed on the norrit and the proportion of the two forms remaining unadsorbed was altered from that of the original solution, as shown by the decrease in the ratio $\frac{I}{F}$. If the forms were adsorbed in proportion to their concentration in the original solution, then the filtrate would be relatively richer in alpha lactose, but actually it is poorer. Furthermore, experiment F showed that when the adsorbed sugar was eluted from the norrit by alcohol the eluate was richer in alpha as shown by the increase in the ratio $\frac{I}{F}$. This demonstrates the preferential adsorption of the alpha form by norrit. Data obtained in a series of other experiments not recorded here can be explained on this basis.

In experiment G lactose in solution was added to a mixture of norrit suspension and alcohol. Practically no adsorption took place and the ratio of the two forms in the filtrate remained unchanged. The previous adsorption of lactose by the norrit does not alter the ratio of alpha to beta in the filtrate when the norrit surface is later freed of lactose by the addition of alcohol as the last step in preparing the mixture. This was demonstrated in experiment H. Experiments A, B, C, D, G, and H show good agreement with the calculated values, for the rotations and also the proportions of alpha and beta lactose in the mixture.

Recovery of lactose added to dried milk and dried whey. Table 4 shows the percentage recovery of specific forms of lactose when added to dried milk

TABLE 3

Adsorption of lactose by norrit, preferential adsorption of the alpha form and elution with alcohol

The suspension of extracted norrit added, contained 2.2 grams. In each experiment of 0.74 gram of anhydrous lactose was made up to 100 ml. and the solutions were polarized at 25° C. in a 4 decimeter tube.

Expt.	Preparation of the solutions	Rotations			Lactose present as		Total lactose in sample %
		I	F	$\frac{I}{F}$	Alpha %	Beta %	
A	Dissolved in water.	1.679 1.680	1.620 1.616	1.036 1.040	40.5 40.9	59.5 59.1	99.3 99.1
B	Dissolved in water, filtered.	1.672 1.675	1.617 1.623	1.034 1.032	40.3 40.1	59.7 59.9	99.1 99.5
C	Dissolved in H ₂ O; sol'n. added to alcohol + H ₂ O; contained 20% alcohol after diluting to mark.	1.648 1.643	1.610 1.612	1.024 1.019	39.3 38.8	60.7 61.2	98.7 99.8
D	Dissolved in H ₂ O; sol'n. added to alcohol + H ₂ O; contained 20% alcohol after diluting to mark; filtered.	1.655 1.635	1.610 1.593	1.028 1.026	39.7 39.5	60.3 60.5	98.7 97.7
E	Dissolved in H ₂ O; sol'n. added to norrit, brought to volume, centrifuged, decanted, filtered.	0.842 0.854	0.858 0.862	0.981 0.991	35.0 36.0	65.0 64.0	52.6 52.8
F	Packed norrit from above extracted with 20% alcohol.	0.760 0.829	0.715 0.767	1.063 1.081	43.3 45.1	56.7 54.9	43.8 47.0
G	Aqueous sol'n. of sugar added to norrit + alcohol; sol'n. contained 20% alcohol diluting to mark.	1.662 1.674	1.606 1.623	1.035 1.031	40.4 40.0	59.6 60.0	98.5 99.5
H	Aqueous sol'n. of sugar added to norrit for adsorption; sugar eluted by adding alcohol before diluting to mark; filtrate contained 20% alcohol.	1.658 1.642	1.612 1.599	1.029 1.027	39.8 39.6	60.2 60.4	98.8 98.0
I	Mixture as made by weighing, calculated.	1.681	1.631	1.031	40.0	60.0	100.0

TABLE 4
Recovery of added lactose
 Lactose added: 1 gm. pure anhydrous Alpha lactose (weighed as hydrate) or
 1 gm. pure anhydrous Beta lactose.

Sample analyzed	Lactose added	Rotations, 4 dm. tube 25° C.			Amount of lactose		Total lactose in product %	Gm. anhydrous lactose in filtrate			Per cent added lactose recovered		
		I	F	I F	As Alpha %	As Beta %		Total	Alpha	Beta	Total	Alpha	Beta
Skim milk 40 (glass)	None	2.75	2.609	1.054	42.4	57.6	47.4	1.1837	0.5019	0.6818
	Alpha	6.37	4.847	1.314	68.6	31.4	2.1991	1.5086	0.6905	100.7
	Beta	4.21	4.874	0.864	23.2	76.8	2.2114	0.5130	1.6984	102.8	101.7
Whey 1 (Alpha)	None	5.96	3.818	1.561	93.6	6.4	69.3	1.7322	1.6213	0.1109
	Alpha	9.57	6.028	1.588	96.3	3.7	2.7349	2.6337	0.1012	100.3	101.2
	Beta	7.46	6.084	1.226	59.8	40.2	2.7603	1.6507	1.1096	102.8	99.9
Whey 2 (Beta)	None	2.57	3.595	0.715	8.1	91.9	65.2	1.6311	0.1321	1.4990
	Alpha	6.19	5.827	1.062	43.2	56.8	2.6437	1.1421	1.5016	101.3	101.0
	Beta	4.03	5.822	0.692	5.8	94.2	2.6414	0.1532	2.4882	101.0	98.9
Whey 67 (Alpha)	None	5.51	3.470	1.588	96.3	3.7	63.0	1.5743	1.5161	0.0582
	Alpha	9.01	5.647	1.596	97.2	2.8	2.5621	2.4904	0.0717	98.8	97.4
	Beta	6.96	5.667	1.228	60.0	40.0	2.5711	1.5437	1.0284	99.7	97.0

TABLE 5

Amount of alpha and beta lactose in selected samples of dried whey and milk

Sample No. and nature	Rotations 4 dm. tube, 25° C.			Amount of lactose		Total anhy- drous lactose in product %
	I	F	$\frac{I}{F}$	%	As Beta	
				As Alpha	%	
51. Whey, beta type	2.58 2.55	3.45 3.47	0.748 0.735	11.4 10.1	88.6 89.9	62.6 63.0
53. Whey, beta type	3.31 3.27	3.84 3.96	0.862 0.826	22.9 19.3	77.1 80.7	69.7 71.9
71. Whey, beta type	3.00 2.97	3.64 3.67	0.824 0.809	20.1 17.6	79.9 82.4	66.1 66.7
58. Whey, beta type	3.37 3.35	3.70 3.76	0.911 0.891	27.9 25.9	72.1 74.1	67.2 68.2
64. Whey, beta type	3.19 3.22	3.63 3.65	0.879 0.882	24.7 25.0	75.3 75.0	65.9 66.2
F. Whey, beta type	3.07 3.13	3.73 3.74	0.823 0.837	19.0 20.4	81.0 79.6	67.7 67.9
82. Modified whey	2.27 2.26	2.20 2.20	1.032 1.027	40.1 39.6	59.9 60.4	39.9 39.9
24. Whey, alpha type, pan condensed	5.40 5.47	3.56 3.58	1.517 1.528	89.2 90.3	10.8 9.7	64.6 65.0
40S. Whey, alpha type, pan condensed	6.13	3.88	1.580	95.5	4.5	70.4
41S. Whey, alpha type, pan condensed	5.81	3.74	1.553	92.8	7.2	67.9
67. Whey, alpha type, pan condensed (old analysis)	5.62 5.60	3.63 3.62	1.548 1.547	92.3 92.2	7.7 7.8	65.9 65.7
65. Whey, alpha type, spray dried	5.09 5.13	3.48 3.51	1.463 1.462	83.7 83.6	16.3 16.4	63.2 63.7
72. Whey, alpha type, spray dried	5.18 5.24	3.43 3.48	1.510 1.506	88.5 88.1	11.5 11.9	62.3 63.2
10. Skimmilk, roll dried	2.70	2.56	1.055	42.5	57.5	46.5
11. Skimmilk, roll dried	2.12	2.02	1.050	42.0	58.0
M20. Skimmilk, roll dried	2.92	2.75	1.062	43.2	56.8	49.9
M40. Skimmilk, roll dried	2.76	2.60	1.062	43.2	56.8	47.2
M60. Skimmilk, roll dried	2.69	2.55	1.055	42.5	57.5	46.3
M80. Skimmilk, roll dried	2.51	2.43	1.033	40.2	59.8	44.1

or whey samples. The recovery of total lactose of each individual form is as good as might be expected. The data in table 4 show an increase in experimental error, when the method is applied to dried milk or whey—as compared to the errors seen in tables 1 and 2 where pure sugars were analyzed. Several additional potential sources of error are introduced in analyzing dried milk and whey.

Alpha and beta lactose in samples of dried milk and whey. Table 5 presents analyses of various typical dried products and shows the wide variations encountered in the relative amounts of alpha and beta lactose, ranging from about 90 per cent in the beta to 95 per cent in the alpha form. The amount of total lactose in the products also showed considerable variation.

SUMMARY

1. A method is described for the determination of the amounts of alpha and beta lactose in dried milk and whey, and also of the total amount of anhydrous lactose in these products.

2. A clear, colorless extract of the product is obtained quickly, using an alcoholic solution of Hg Cl_2 as protein precipitant, and norrit as a decolorizing agent. The filtrate is polarized as soon as possible and again after standing. The relative amounts of alpha and beta lactose are computed from the change in optical rotation. Total lactose is calculated from the final (equilibrium) rotation and sample weight.

3. Alpha and beta lactose gave satisfactory results when analyzed by this method, as did also dried milk and whey, alone and with additions of alpha and beta lactose.

4. The composition of the lactose in dried whey was shown to range from 90 per cent beta to 95 per cent alpha, on the anhydrous basis.

5. Norrit adsorbs lactose from aqueous solution, the alpha form being adsorbed preferentially. Lactose is eluted from the norrit by 10 to 20 per cent alcohol.

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FACTORS AFFECTING THE ACTIVITY AND HEAT RESISTANCE
OF SWISS CHEESE STARTER CULTURES. III. EFFECT
OF VARIATIONS IN TIME AND TEMPERATURE
OF INCUBATION AND OF STORAGE ON
ACTIVITY OF CULTURES*

H. J. PEPPLER AND W. C. FRAZIER

Department of Agricultural Bacteriology, University of Wisconsin

The variation permissible in methods of preparation and storage of cultures of lactic acid bacteria without causing reduction in their activity remains a practical problem to the dairy manufacturer. In a previous paper Elliker and Frazier (1) reported the influence of incubation temperature on heat resistance of certain starter organisms for Swiss cheese; in their experiments, incubation periods common in actual practice were employed. The present study was undertaken to ascertain how much variation in time and temperature of incubation was permissible without loss in activity of the starter organisms. The effect of these same variations on heat resistance of cultures will be discussed in the following paper of this series.

A definite relationship between the titratable acidity of starter cultures grown at a constant temperature and the quality of Swiss cheese was observed by Frazier and associates (3). The most effective milk starter cultures of *Lactobacillus helveticus*, Strain 39a, had titratable acidities of 1.00 to 1.09 per cent (as lactic acid) after growth at 37.5° to 39° C. for 12 hours. In experiments with cultures of *Streptococcus thermophilus*, Strain C-3, best results were noted when milk cultures showed titratable acidities of 0.70 to 0.75 per cent after 12 hours at 37° C. Thus the preparation of starter cultures requires controlled temperatures and periods of incubation for production of the desired rate of lactic acid fermentation in the cheese in the press. Information bearing on the methods or procedures of preparing active starter cultures should be helpful not only to the makers of Swiss cheese but also to all who handle active cultures of thermoduric lactic acid bacteria.

EXPERIMENTAL

Pure cultures of *Lactobacillus helveticus*, Strain 39aW, and *Streptococcus thermophilus*, Strain C-3, were grown at constant temperatures in freshly autoclaved ten per cent reconstituted skimmilk. After seven to nine consecutive transfers at a given temperature, the activity of each mother culture, as evidenced by amount of growth and fermentation, was de-

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terminated. For bulk culture, an inoculum of 0.25 per cent, from a mixture of equal parts of mother culture and a two per cent solution of sodium citrate, was transferred into 400 ml. of fresh skimmilk medium. Bulk cultures were incubated for 12 hours, and usually at the same temperature as that previously used for the mother culture. The amount of growth in bulk cultures was determined by direct microscopic count of living cells, according to the method of Frazier and Boyer (2). Acidity of bulk cultures was measured by titration, and pH was calculated from the potentials shown by the quinhydrone electrode. Direct counts were made also of mother cultures after incubation; in some studies, the acidities of mother cultures were used for comparison. Representative experiments are summarized briefly.

Influence of incubation time of mother cultures grown at 37° C. on the activity of 12-hour bulk cultures. In a series of several experiments the cultures were incubated at 37° C. and transferred every 12, 24, 36, 48 and 168 hours for seven to nine successive transfers. The recommended period for incubation of Swiss cheese starters has been 12 to 14 hours at 37° C., so that their maturity could be judged by the acidity produced in that time. A comparison of activity of 12-hour bulk cultures prepared from mother cultures incubated at 37° for periods longer than those commonly employed is made in table 1; arithmetic averages of several experiments are given.

TABLE 1

Differences in activity of bulk cultures after 12 hours at 37° C.; inoculated with mother cultures of Lactobacillus helveticus or Streptococcus thermophilus grown at 37° C. and transferred every 12, 24, 36, 48 or 168 hours*

Mother culture		Activity of bulk culture		
Incubation time	Direct count	Direct count	Titratable acidity	pH
hours	millions/ml.	millions/ml.	per cent	
<i>L. helveticus</i>				
12	1,013	1,294	1.05	4.10
24	1,041	1,249	1.04	4.18
36	796	703	0.77	4.42
48	714	464	0.63	4.70
168	519	2	0.26	5.90
<i>Str. thermophilus</i>				
12	1,032	1,079	0.82	4.41
24	1,017	1,363	0.81	4.46
36	797	597	0.73	4.51
48	732	380	0.63	4.67
168	410	19	0.27	5.88

* Seven to nine successive transfers.

Cultures of *L. helveticus* or *Str. thermophilus* after numerous transfers at 37° C. every 12 or 24 hours were similar in growth and fermentation in 12-

hour bulk cultures. Periods of incubation greater than 24 hours, however, resulted in a decrease in the numbers of cells in both mother cultures and 12-hour bulk cultures, while the acidities developed in bulk cultures decreased progressively as the age of mother culture increased.

Influence of incubation time of mother cultures grown at 40°, 42°, or 45° C. on the activity of 12-hour bulk cultures. Since relatively short periods (12 to 24 hours) of incubation of mother cultures grown at 37° C. resulted in no marked change in the activity of 12-hour bulk cultures, higher temperatures of incubation for similar periods were employed. It might be expected that the incubation of mother cultures at temperatures above 37° and for periods greater than 24 hours would seriously decrease acid production in 12-hour bulk culture. The results of preliminary experiments substantiated this belief.

TABLE 2

Differences in activity of bulk cultures after 12 hours at 40°, 42°, or 45° C.; inoculated with mother cultures of Lactobacillus helveticus or Streptococcus thermophilus grown at 40°, 42°, or 45° C. and transferred every 12 and 24 hours

Mother culture		Activity of bulk culture			Incubation temperature of all cultures
Incubation time	Direct count	Direct count	Titratable acidity	pH	
hours	millions/ml.	millions/ml.	per cent		°C.
<i>L. helveticus</i>					
12	880	1,070	1.08	4.01	40
24	871	967	0.99	4.21	40
12	1,579	1,309	0.97	4.10	42
24	864	1,028	0.93	4.19	42
12	0.46	5.13	45
24	0.68	4.58	45
<i>Str. thermophilus</i>					
12	739	828	0.70	4.63	40
24	634	733	0.70	4.64	40
12	1,348	1,211	0.76	4.35	42
24	1,444	923	0.74	4.38	42
12	0.70	4.66	45
24	0.67	4.65	45

The effect of cultivation of mother cultures at 40°, 42°, and 45° C. for successive 12- and 24-hour periods upon the activity of 12-hour bulk cultures is shown in table 2. Mother cultures of *L. helveticus* grown at 40° or 42° C. for 12 hours were slightly more active in 12-hour bulk culture than the 24-hour mother cultures; however, incubation at 45° gave irregular data, apparently due to the close approach of the incubation temperature to the maximum temperature of growth of this organism. A comparison of the data on the bulk cultures prepared from 12- and 24-hour mother cultures of *Str. thermophilus* provides no conclusive evidence that the 12-hour mother

cultures at a given temperature of incubation are superior in fermentation or growth to the 24-hour mother cultures carried at the same temperature.

Differences in mother cultures as shown by the activity of bulk cultures in the initial phases of the growth cycle. Although differences in acidity and total cell counts could not be demonstrated in 12-hour bulk cultures, the possibility that differences in growth and fermentation existed during the first few hours after inoculation was investigated. A comparison of bulk cultures, made 2.5 to 3 hours after inoculation, appears in table 3.

TABLE 3

Comparison of amount of growth and fermentation of bulk cultures shortly after inoculation with mother cultures which had been transferred at different temperatures every 12 and 24 hours

Mother culture		Activity of bulk culture				
Incubation		Incubation		Direct count of inoculum	Direct count after incubation	Drop in pH
Time	Temp.	Temp.	Time			
hours	°C.	°C.	hours	millions/ml.	millions/ml.	
<i>L. helveticus</i>						
12	37	37	2.5	1.82	8.42	0.06
24	37	37	2.5	1.60	19.1	0.07
12	37	42	2.5	1.33	16.6	0.13
24	37	42	2.5	1.08	12.7	0.11
12	40	40	3.0	2.02	31.9	0.22
24	40	40	3.0	2.21	15.9	0.04
12	42	42	3.0	0.07
24	42	42	3.0	0.02
<i>Str. thermophilus</i>						
12	37	37	2.5	.810	109.	0.26
24	37	37	2.5	.916	77.0	0.20
12	40	40	2.5	.410	45.0	0.34
24	40	40	2.5	.430	34.7	0.16
12	40	43	2.5	.410	77.4	0.46
24	40	43	2.5	.430	59.9	0.27
12	42	42	2.5	0.44
24	42	42	2.5	0.22

Mother cultures of *L. helveticus*, incubated at 37° for 12 and 24 hours, were equally as active in 2.5-hour bulk cultures at 37° as in similar subcultures at 42°. Cultures of *Str. thermophilus* handled in a similar manner were shown to be equivalent in activity when mother cultures were grown at 37° for 12 or 24 hours. Cultures of both species of bacteria grown above 37° for 12 or 24 hours exhibited greatest variations in activity when the amounts of decrease in pH were compared. The differences in activity of cultures observed during the early hours of incubation were absent at the time 12-hour bulk cultures were compared. The capacity of 24-hour cultures to overtake the 12-hour mother cultures was more apparent with *Str. thermophilus* than *L. helveticus*. It may be related to the increased fer-

menting capacity of old cells observed by Rahn *et al.* (6) with old cells of *Str. lactis* beginning to multiply in a fresh medium.

Influence of low storage temperatures after the incubation of mother cultures upon the activity of 12-hour bulk cultures. The high degree of activity exhibited by mother cultures transferred at consecutive 24-hour intervals suggested that relatively short periods of incubation near the optimum temperature of growth for these bacteria could be used, followed by storage at temperatures below the optimum for growth. Mother cultures were incubated at 37° for 9, 12, or 16 hours, placed promptly at 0°, 20°, and 30° C., respectively, and stored until each culture was 24 hours old. The activities of bulk cultures grown at 37° for 12 hours are compared in table 4. Mother cultures of *L. helveticus* were stored at 0° and 20° until 24 hours old, following an initial incubation period of 12 to 16 hours at 37°, without reduction in growth or fermentation. Storage at 30°, however, resulted in decreased titratable acidities of the bulk cultures. Although Kopeloff *et al.* (4) reported that storage of other lactobacilli at 20° was less harmful than at lower temperatures, the data shown here indicate no marked differences in viability of *L. helveticus* during storage at 0° or 20° C.

TABLE 4

Differences in activity of bulk cultures after 12 hours at 37°C.; inoculated with mother cultures of Lactobacillus helveticus or Streptococcus thermophilus grown at 37°C. for 9, 12 or 16 hours and then stored at 0°, 20°, or 30°C. until 24 hours old

Mother cultures					Activity of bulk cultures		
Incubation time at 37°C.	Storage		pH	Direct count	Direct count	pH	Titratable acidity
	Time	Temp.					
hours	hours	°C.		millions/ml.	millions/ml.		per cent
<i>L. helveticus</i>							
9	15	0	4.55	674	1,069	4.54	0.76
12	12	0	4.23	727	863	4.47	0.85
16	8	0	4.09	739	876	4.45	0.85
12	12	20	4.21	887	790	4.47	0.87
16	8	20	4.14	1,015	879	4.42	0.87
12	12	30	4.02	623	4.50	0.75
16	8	30	3.93	830	4.47	0.75
<i>Str. thermophilus</i>							
9	15	0	4.78	982	744	4.56	0.70
12	12	0	4.51	892	594	4.58	0.71
16	8	0	4.39	792	904	4.57	0.71
12	12	20	4.46	927	1,299	4.67	0.67
16	8	20	4.41	1,156	576	4.65	0.68
12	12	30	4.36	1,336	1,014	4.55	0.71
16	8	30	4.33	744	746	4.55	0.71

Cultures of *Str. thermophilus*, when treated in a manner similar to that

described for *L. helveticus*, were equally as active in bulk culture after storage at 0° and 20° C. as they were after being held at 30°. The initial incubation time of mother cultures before storage, whether 9, 12, or 16 hours, had no marked influence on the direct counts or acidities of the bulk cultures. Results of experiments (5) analogous to the one just described, except that bulk cultures were incubated at 30°, confirmed these observations and revealed that *L. helveticus* and *Str. thermophilus* could be stored at 0°, 10° or 20° C. for similar periods without reduction in activity.

Since a short storage period was harmless to either *L. helveticus* or *Str. thermophilus*, the effect of variations in storage time on growth and fermentation of mother cultures was studied. Cultures held at 42° for 12, 16, or 24 hours were continued at 20° for different periods. According to results shown in table 5, the incubation and storage time of mother cultures of *L. helveticus* and *Str. thermophilus* could be varied considerably without altering significantly the numbers of cells and titratable acidities of 12-hour bulk cultures. Cultures incubated at 42° for 12 or 24 hours could be stored

TABLE 5

Differences in activity of bulk cultures after 12 hours at 42°C.; inoculated with mother cultures of Lactobacillus helveticus or Streptococcus thermophilus grown at 42°C. for 12, 16, or 24 hours and then stored at 20°C. until 24, 48 or 96 hours old

Mother culture				Activity of bulk culture		
Ineubation time at 42°C.	Storage at 20°C.	pH	Direct count	Direct count	pH	Titratable acidity
hours	hours		millions/ml.	millions/ml.		per cent
<i>L. helveticus</i>						
12	12	4.15	516	965	4.27	0.95
16	8	4.00	659	766	4.25	0.93
12	36	3.89	893	1,013	4.27	0.98
12	84	3.78	784	1,006	4.14	1.01
24	24	3.71	886	889	4.35	0.93
24	72	3.69	601	641	4.30	0.93
12	none	1,579	1,309	4.10	0.97
24	none	864	1,028	4.19	0.93
<i>Str. thermophilus</i>						
12	12	4.47	541	856	4.44	0.75
16	8	4.39	592	505	4.55	0.71
12	36	4.29	493	1,135	4.64	0.61
12	84	4.24	807	1,049	4.44	0.74
24	24	4.20	462	1,029	4.67	0.64
24	72	4.06	849	881	4.40	0.72
12	none	1,348	1,211	4.35	0.76
24	none	1,444	923	4.38	0.74

at 20° an additional 84 or 72 hours, respectively, without reduction in their activity. Storage under these conditions produced mother cultures equiva-

lent in bulk culture activity to cultures transferred every 12 or 24 hours near the optimum growth temperature. During storage at 20° a substantial number of cells die off, and mother cultures increase slowly in acidity. At this temperature, however, the effect of the developed acidity on the enzyme system of the majority of cells is probably diminished. According to Sarkaria and Hammer (7), true lactic acid bacteria grown in milk at different temperatures produced no measurable changes other than variations in total micro-population and acidity.

DISCUSSION

The results show that active cultures of *L. helveticus* and *Str. thermophilus* could be prepared with considerable latitude in incubation time and temperature. Variations within limits in methods of incubation of stock cultures can be employed with assurance that satisfactory growth and fermentation of cultures will be maintained. When large masses of cells, or bulk cultures, are required, similar variations in incubation time and temperature could be employed; and, in addition, if only a portion of the bulk culture is used, the remainder can be stored for short periods at low temperatures without loss in vitality. Thus time may be saved by the preparation of large quantities of bulk culture and punctuality in transferring may not be necessary, even though starter cultures may be needed every day or twice a day.

The results obtained in this study may not indicate the effects of these variations on the heat resistance of starter bacteria. This phase of the problem will be discussed in the following paper of this series.

SUMMARY

1. For mother cultures transferred successively at either 37°, 40°, or 42° C., the incubation time of *L. helveticus* and *Str. thermophilus* may be varied as much as 12 hours beyond an initial incubation period of 12 hours without harmful effect on culture activity. Cultures transferred every 12 or 24 hours produced similar populations and acidities when 12-hour bulk cultures made from them were compared at 37°.

2. Continuous incubation at 45° with transfers every 12 or 24 hours produced cultures of *Str. thermophilus* which were equivalent in activity to those grown at either 37°, 40°, or 42° C. Cultures of *L. helveticus* grew irregularly and poorly after successive transfers at 45°.

3. After incubation at 37° from 12 to 16 hours, or at 42° from 12 to 24 hours, cultures of *L. helveticus* could be stored at 0° to 20° C. until they were 96 hours old without reduction in their activity.

4. Cultures of *Str. thermophilus* incubated at 37° from 9 to 16 hours, or at 42° from 12 to 24 hours, could be continued at 0° or 20° C. until they

were 96 hours old without becoming less active than cultures transferred every 12 hours at 37° or 42° C.

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FACTORS AFFECTING THE ACTIVITY AND HEAT RESISTANCE
OF SWISS CHEESE STARTER CULTURES. IV. EFFECT
OF VARIATIONS IN TIME AND TEMPERATURE OF
INCUBATION AND OF STORAGE ON HEAT
RESISTANCE OF CULTURES*

H. J. PEPPLER AND W. C. FRAZIER

Department of Agricultural Bacteriology, University of Wisconsin

Improved methods of handling and preparing starter cultures of thermophilic lactic acid bacteria are useful to dairy manufacturers, especially to makers of Swiss cheese to whom the activity and heat resistance of the bacteria are of great importance. There is frequently a desire to modify the method of preparation of starter cultures, but the uncertainty of the effect the change may have on the vitality of the bacteria has limited attempts to vary customary procedures.

The present study was undertaken to determine to what extent time and temperature of incubation and of storage could be varied without loss in satisfactory activity and heat resistance of *Lactobacillus helveticus* and *Streptococcus thermophilus*, bacteria commonly used in dairy manufacture.

Previous investigations by us (13) have shown that incubation periods at a constant temperature near the optimum for growth, or in combination with storage at low temperatures, may be varied considerably without reduction of vitality of *L. helveticus* and *Str. thermophilus*. In these experiments the heat resistance of mother cultures was not determined. Brief studies by Elliker (4) demonstrated that cultures grown at 37° or 40° C. for 12 hours and promptly stored for the same time at 10° were equally as heat resistant as cultures transferred every 12 hours at 37° or 40° C.

Elliker and Frazier (5) observed that 12 and 16-hour cultures of *L. helveticus* developed better after a severe heat treatment than 7 or 8-hour cultures. They also established that greater resistance was exhibited by cultures incubated at 37° or 40° C. than by those grown at 30°, 35°, or 42° C. Six, 12 and 16-hour cultures of *Str. thermophilus* were equal in heat resistance when grown at 37°, but differences appeared when incubation was at 40°. Cultures grown at 30°, 35°, or 37° C. were more heat resistant than those incubated at 40° or 42° C.

Comprehensive investigations of factors affecting heat resistance of non-sporeforming bacteria appear in the literature. Such factors as temperature of incubation, age of culture, character of culture medium, and condi-

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tions of the heat treatment have been reviewed and studied by Anderson and Meanwell (1), Brown and Peiser (2), Claydon (3), Elliker (4), Elliker and Frazier (5), Fay (7), Peppler (12), and Robertson (14).

EXPERIMENTAL

Lactobacillus helveticus, Strain 39aW, and *Streptococcus thermophilus*, Strain C-3, the organisms used in studies by Elliker and Frazier (5, 6) and by us (13), were employed in the major portion of this investigation. In part of the work the heat resistance of the same strain of *L. helveticus* and Strain Mc of *Str. thermophilus*, both grown in symbiosis with a film yeast, *Candida krusei*, commonly called a "mycoderm," were compared with unasociated cultures of these bacteria.

Except for minor modifications the methods and apparatus used by Elliker and Frazier (5) were adopted. Mother cultures were grown in freshly autoclaved reconstituted skimmilk, designated as the "normal" medium. Since the heat resistance of *L. helveticus* is influenced by the quality of the medium, as shown by Elliker and Frazier (6), the organism also was grown in a "fortified" medium composed of freshly autoclaved reconstituted skimmilk and 0.1 per cent Neopeptone. *Str. thermophilus* was carried in the fortified medium in some studies. After seven to nine consecutive culture generations, final transfers were made to a larger quantity of medium to facilitate removal of samples for heat treatment and determinations of acidity. These cultures were designated as inoculating cultures. Heat resistance of cultures was measured in terms of rapid growth and acid production of subcultures in the normal skimmilk medium following a heat treatment simulating that encountered during Swiss cheese manufacture. Subcultures of *L. helveticus* were heated at 60° or 62° C. for 30 minutes; subcultures of *Str. thermophilus* were subjected to 62° or 64° C. for the same period. In most studies the higher temperature mentioned for each organism was adopted to emphasize differences in the activity of cultures. Plate counts were substituted for the direct microscopic method used by Elliker and Frazier to determine the number of viable cells directly before and after heating, and the rate of growth following the heat treatment. Plates were poured in triplicate using the medium of Kulp and White (9). Heat-treated subcultures were incubated at 37° to avoid the long chains which these bacteria form at higher temperatures. The amount of decrease in pH of the incubated culture during a definite period after heating was used as a measure of activity of surviving cells.

Influence of 12 and 24-hour successive transfers of mother cultures on heat resistance. The temperatures selected for incubation of the mother cultures of *L. helveticus* and *Str. thermophilus* were 37° and 40° C., since it had been found that heat resistant cultures were produced at both tem-

peratures, although the higher temperature was better under some conditions. Mother cultures of each organism were transferred every 12 and 24 hours. *L. helveticus* was grown in two different media, reconstituted skim-milk, or "normal" medium, and the same medium with "Neopeptone" added. Cultures of *Str. thermophilus* were grown in the fortified medium when it was observed that cultures carried in the normal medium developed a low resistance to heat.

TABLE 1

Influence of kind of culture medium and of successive transfers¹ of mother cultures of Lactobacillus helveticus and Streptococcus thermophilus on the activity of subcultures at 37° C. following heat treatment

Inoculating cultures			Heat-treated subcultures			
Incubation time and temperature	Kind of medium	Titratable acidity	Plate count before heating	Plate count after heating	Temp. of heating	Drop in pH seven hours after heating
		per cent	millions/ml.	millions/ml.	°C.	
<i>L. helveticus</i>						
12 hrs. 37°	normal ²	0.85	2.34	2.17	60	0.39
12 hrs. 37°	fortified ³	0.89	2.47	2.23	60	0.48
24 hrs. 37°	normal	1.27	2.23	1.74	60	0.36
24 hrs. 37°	fortified	1.27	2.50	1.99	60	0.47
12 hrs. 40°	normal	1.13	2.66	2.46	62	0.53
12 hrs. 40°	fortified	1.09	2.08	2.02	62	0.53
24 hrs. 40°	normal	1.47	2.44	2.35	62	0.49
24 hrs. 40°	fortified	1.25	1.75	1.61	62	0.44
<i>Str. thermophilus</i>						
12 hrs. 37°	normal	0.71	0.614	0.407	62	1.22
24 hrs. 37°	normal	0.88	0.560	0.357	62	1.16
12 hrs. 40°	normal	0.72	0.585	0.220	62	0.98
12 hrs. 40°	fortified	0.90	0.720	0.490	62	1.26
24 hrs. 40°	normal	0.87	0.290	0.069	62	0.95
24 hrs. 40°	fortified	0.91	0.405	0.232	62	0.85

¹ Seven to nine consecutive transfers.

² Reconstituted skimmilk.

³ Neopeptone-skimmilk medium.

The effect of successive transfers on the activity of heat-treated subcultures, as shown in table 1, varies not only with incubation temperature but also the quality of the medium. In order to limit the extensive data concomitant with hourly determinations of plate counts and acidity after heating, the plate counts directly before and after the heat treatment are reported, together with the amount of decrease in pH after six or seven hours. Mother cultures of *L. helveticus* grown in a fresh skimmilk medium at 40°, were more heat resistant than similar cultures developed at 37°. But in the fresh Neopeptone-skimmilk medium no significant differences in activity

after the heat treatment were exhibited by cultures grown either at 37° from 12 to 24 hours or at 40° for 12 hours. When *Str. thermophilus* was transferred every 12 and 24 hours at 37°, the cultures were equivalent to each other and more heat resistant than similar cultures carried at 40°. Growth in a fortified medium, however, provided 12-hour mother cultures at 40° which were equally as active after heating as those grown at 37°.

Influence of time and temperature of storage of mother cultures after incubation on heat resistance. Since the heat resistance of mother cultures was unaffected by certain limited variations in the incubation time during growth at a constant temperature, it was believed that such active cultures could be stored at low temperatures for limited periods without a reduction in heat resistance. *L. helveticus* and *Str. thermophilus* were incubated at 37° and 40° C. for periods of 6 to 24 hours and then promptly placed at 20°, 12°, or 4° C. for different periods of time. A constant temperature water bath was used for storage at 20°; an icebox having an average temperature of 12°, and a refrigerator with an average temperature of 4° provided the other means of storage.

The effect of storage time and temperature upon heat resistance of *L. helveticus* initially incubated at 37° for 12 or 24 hours is shown in table 2. Storage at 12° resulted in cultures with greater activity after a severe heat treatment than storage at 20° or 4° C. Storage time could be varied widely without significant reduction in activity of heat-treated subcultures. When *L. helveticus* was held at 37° for 12 hours, cultures could be stored at 12° for 60 hours and still be as heat resistant as the most active culture transferred every 12 hours at 37° C. Cultures incubated 24 hours at 37° and stored 48 hours at 12° were equally as resistant as those just described. When cultures were grown in a fresh Neopeptone-skimmilk medium before storage, they were more active after heating than similar cultures in the normal medium, but storage time was not prolonged beyond limits previously observed.

Storage of *Str. thermophilus*, after incubation at 37°, by methods similar to those just described for *L. helveticus*, resulted in heat resistant cultures at all low storage temperatures used, but, as the data in table 3 indicate, storage time at 12° could be varied more widely than at 4° or 20° C. Cultures which had been carried in a skimmilk medium with added Neopeptone showed more activity after the heat treatment than cultures which had been carried in the normal skimmilk medium. When *Str. thermophilus* was grown in the fortified medium, cultures carried at 37° for 12 or 24 hours followed by storage at 12° for 84 or 24 hours, respectively, as well as cultures grown 12 hours at 37° and continued for 36 hours at 4° C. were equivalent in activity after heating to cultures transferred serially at 37° in the normal medium.

According to data of table 4, *L. helveticus* incubated at 40° from 8 to 15 hours could be held for two days at low temperatures without decrease in heat resistance. When mother cultures were carried in the normal medium, incubation periods of 10 to 15 hours at 40° could be followed by 38 and 33 hours, respectively, at 20° or 12° C. In the medium with added Neopeptone, incubation periods at 40° for as little as 8 hours did not cause a reduction in heat resistance. The most active cultures in these experiments were equivalent in heat resistance to cultures transferred serially at either 37° or 40° C.

Previous investigations demonstrated that cultures of *Str. thermophilus* grown at 40° were less active after heat treatment than those carried at 37°. It could be expected that cultures stored at low temperatures after incubation at 40° would be no better than cultures transferred continuously at

TABLE 2

Influence of kind of culture medium, incubation time at 37° C., and storage time and temperature of mother cultures of Lactobacillus helveticus on the activity of subcultures at 37° C. following heat treatment

Inoculating cultures				Heat-treated subcultures			
Incubation time at 37°	Storage time and temperature	Kind of medium	Titrat-able acidity	Plate count before heating	Plate count after heating	Temp. of heating	Drop in pH seven hours after heating
hours			per cent	millions/ml.	millions/ml.	°C.	
12	none	normal	0.85	2.34	2.17	60	0.39
	none	fortified	0.89	2.47	2.23	60	0.48
	36 hrs. 20°	normal	1.04	1.14	0.85	60	0.36
	36 hrs. 20°	fortified	1.07	1.45	1.21	60	0.39
	36 hrs. 12°	normal	0.97	2.48	2.55	62	0.50
	36 hrs. 12°	fortified	0.98	2.69	2.52	62	0.53
	12 hrs. 4°	normal	0.96	3.15	2.65	62	0.38
	36 hrs. 4°	normal	0.92	2.99	2.41	62	0.39
	84 hrs. 20°	normal	1.20	2.02	1.96	60	0.22
	84 hrs. 20°	fortified	1.30	2.17	2.04	60	0.28
	60 hrs. 12°	normal	1.22	2.02	2.16	62	0.46
	84 hrs. 12°	normal	1.06	1.89	1.69	62	0.37
	60 hrs. 4°	normal	0.97	2.62	2.45	62	0.43
	156 hrs. 4°	normal	0.91	2.93	2.57	62	0.31
24	none	normal	1.27	2.23	1.74	60	0.36
	none	fortified	1.27	2.50	1.99	60	0.47
	24 hrs. 20°	normal	2.90	2.56	60	0.36
	24 hrs. 20°	fortified	1.45	2.86	2.73	60	0.39
	24 hrs. 12°	normal	1.29	2.12	2.16	62	0.46
	24 hrs. 12°	fortified	1.21	2.04	1.79	62	0.41
	48 hrs. 12°	normal	1.46	2.26	2.21	62	0.46
	48 hrs. 4°	normal	1.41	3.20	2.74	62	0.43
	72 hrs. 20°	normal	1.35	1.81	1.28	60	0.22
	72 hrs. 20°	fortified	1.44	1.67	1.61	60	0.30
	72 hrs. 12°	normal	1.42	3.15	2.63	62	0.42
	144 hrs. 4°	normal	1.25	2.82	2.52	62	0.26

40°. This presumption was substantiated by the results presented in table 5. Only cultures carried in a medium better than fresh reconstituted skim-milk were as active after heating as the most heat resistant cultures developed at 37°. Cultures incubated in the fortified medium at 40° from 6 to

TABLE 3

Influence of kind of culture medium, incubation time at 37° C., and storage time and temperature of mother cultures of Streptococcus thermophilus on the activity of subcultures at 37° C. following heat treatment

Inoculating cultures				Heat-treated subcultures			
Incuba- tion time at 37°	Storage time and tempera- ture	Kind of medium	Titrat- able acidity	Plate count before heating	Plate count after heating	Temp. of heat- ing	Drop in pH six hours after heat- ing
hours			per cent	millions/ml.	millions/ml.	°C.	
12	{ none	normal	0.72	0.614	0.407	62	1.05
	{ 36 hrs. 20°	normal	0.82	0.640	0.482	62	1.04
	{ 84 hrs. 20°	normal	1.05	0.750	0.378	62	1.12
	{ 156 hrs. 20°	normal	1.01	0.414	0.309	62	0.85
	{ 12 hrs. 12°	normal	0.74	0.727	0.060	64	0.85
	{ 12 hrs. 12°	fortified	0.88	0.809	0.395	64	1.04
	{ 36 hrs. 12°	normal	0.76	0.600	0.155	64	0.88
	{ 36 hrs. 12°	fortified	0.91	0.635	0.133	64	1.13
	{ 84 hrs. 12°	normal	0.84	0.550	0.025	64	0.99
	{ 84 hrs. 12°	fortified	0.94	0.485	0.074	64	1.09
	{ 156 hrs. 12°	normal	0.83	0.675	0.155	64	0.78
	{ 156 hrs. 12°	fortified	0.96	0.780	0.174	64	0.99
	{ 12 hrs. 4°	normal	0.73	0.770	0.043	64	0.53
	{ 12 hrs. 4°	fortified	0.89	0.985	0.188	64	1.02
	{ 36 hrs. 4°	normal	0.68	0.680	0.080	64	0.91
	{ 36 hrs. 4°	fortified	0.82	0.770	0.229	64	1.13
	{ 60 hrs. 4°	normal	0.74	0.612	0.103	64	0.50
10	{ 14 hrs. 12°	normal	0.70	0.724	0.110	64	0.85
	{ 14 hrs. 12°	fortified	0.82	0.867	0.380	64	1.11
24	{ none	normal	0.84	0.560	0.357	62	1.00
	{ 24 hrs. 20°	normal	0.86	0.518	0.352	62	0.96
	{ 72 hrs. 20°	normal	1.05	0.621	0.251	62	0.97
	{ 24 hrs. 12°	normal	0.83	0.585	0.070	64	0.74
	{ 24 hrs. 12°	fortified	0.95	0.590	0.250	64	1.05
	{ 72 hrs. 12°	fortified	1.02	0.940	0.337	64	0.57
	{ 48 hrs. 4°	normal	0.89	0.560	0.235	64	0.09

7 hours, and stored at either 12° or 4° C. from 42 to 41 hours, respectively, exhibited a remarkably high heat resistance. The increased resistance of *L. helveticus* and *St. thermophilus* after growth in fortified skimmilk media may, in a few instances, be due to greater numbers of cells surviving the heat treatment. In most instances marked differences in plate counts of survivors were not observed, regardless of the method of comparison used. Whether increases in heat resistance were the result of a greater accumula-

tion of mature, heat resistant cells in a favorable medium than in a poor medium, as Elliker and Frazier (5) suggest, or whether the growth stimulants merely improve the general healthiness of the cells was not revealed by this investigation.

TABLE 4

Influence of kind of culture medium, incubation time at 40° C., and storage time and temperature of mother cultures of Lactobacillus helveticus on the activity of subcultures at 37° C. following heat treatment at 62° C. for 30 minutes

Inoculating cultures				Heat-treated subcultures		
Incubation time at 40°	Storage time and temperature	Kind of medium	Titrat-able acidity	Plate count before heating	Plate count after heating	Drop in pH seven hours after heating
hours			per cent	millions/ml.	millions/ml.	
10	14 hrs. 20°	normal	1.05	2.42	2.15	0.49
10	38 hrs. 20°	normal	1.21	2.66	2.50	0.46
15	9 hrs. 20°	normal	1.27	2.02	2.50	0.46
15	33 hrs. 20°	normal	1.34	3.10	2.56	0.46
10	14 hrs. 12°	normal	0.87	2.76	2.18	0.48
10	14 hrs. 12°	fortified ¹	1.13	2.78	2.67	0.59
12	12 hrs. 12°	normal	1.03	2.37	2.30	0.51
12	12 hrs. 12°	fortified ¹	1.13	2.39	2.48	0.48
8	40 hrs. 12°	normal	0.86	2.36	1.83	0.36
8	40 hrs. 12°	fortified ¹	1.03	2.71	2.49	0.51
10	38 hrs. 12°	normal	1.00	2.42	2.21	0.52
15	33 hrs. 12°	normal	1.29	2.28	2.26	0.50
8	64 hrs. 12°	normal	0.95	2.45	2.12	0.42
15	57 hrs. 12°	normal	1.29	2.94	2.39	0.39
10	14 hrs. 4°	normal	0.92	2.26	2.28	0.37
15	9 hrs. 4°	normal	1.25	1.96	2.44	0.48
9	39 hrs. 4°	normal	0.87	2.63	2.09	0.44
9	39 hrs. 4°	fortified ¹	1.00	2.57	2.23	0.53
10	38 hrs. 4°	normal	0.92	2.39	2.25	0.37
15	33 hrs. 4°	normal	1.22	2.58	2.99	0.37
12	none	normal	1.13	2.66	2.46	0.39
12	none	fortified ²	1.09	2.08	2.02	0.48
24	none	normal	1.47	2.44	2.35	0.36
24	none	fortified ²	1.25	1.75	1.61	0.47

¹ Malt extract—skimmilk medium.

² Neopeptone—skimmilk medium.

Influence of a film yeast ("mycoderm") on heat resistance of bacteria grown in symbiosis with it. Several cultures in which the thermoduric lactic acid bacteria are associated with a film yeast, *Candida krusei*, sometimes called a "mycoderm," are used commonly by makers of Swiss cheese. In such cultures the "mycoderm" is known to increase viability of bacteria by reduction of the acidity (8, 11), and with some species of bacteria the production of lactic acid is stimulated (8, 10). Reports concerning the effect of a film yeast on thermal resistance of these bacteria have not been found. Comparisons of heat resistance were made between pure cultures of bacteria and the same cultures associated with a film yeast. *L. helveticus*, Strains 39aW and 39aW-my, and *Str. thermophilus*, Strains Mc and Mc-my,

TABLE 5

Influence of kind of culture medium, incubation time at 40° C., and storage time and temperature of mother cultures of Streptococcus thermophilus on the activity of subcultures at 37° C. following heat treatment at 64° C. for 30 minutes

Inoculating cultures				Heat-treated subcultures		
Incuba- tion time at 40°	Storage time and temperature	Kind of medium	Titrat- able acidity	Plate count before heating	Plate count after heating	Drop in pH six hours after heating
hours			per cent	millions/ml.	millions/ml.	
8	16 hrs. 20°	normal	0.75	0.655	0.025	0.27
12	12 hrs. 20°		0.79	0.520	0.025	0.27
15	9 hrs. 20°		0.83	0.535	0.015	0.29
8	40 hrs. 20°		0.81	0.570	0.065	0.52
15	33 hrs. 20°		0.88	0.588	0.150	0.57
8	40 hrs. 12°		0.72	0.589	0.153	0.66
15	33 hrs. 12°		0.80	0.545	0.082	0.46
6	42 hrs. 12°		0.66	0.653	0.064	0.53
8	64 hrs. 12°		0.77	0.595	0.111	0.37
15	57 hrs. 12°		0.84	0.555	0.100	0.37
8	40 hrs. 4°		0.68	0.587	0.253	0.84
15	33 hrs. 4°		0.79	0.661	0.031	0.28
7	41 hrs. 4°		0.58	0.530	0.030	0.48
8	16 hrs. 4°		0.71	0.520	0.060	0.60
15	9 hrs. 4°		0.82	0.428	0.044	0.41
8	64 hrs. 4°		0.65	0.730	0.044	0.52
12	60 hrs. 4°		0.73	0.580	0.042	0.56
6	42 hrs. 12°	fortified ¹	0.82	0.645	0.333	1.25
7	41 hrs. 4°		0.78	0.780	0.268	1.14
12	none		0.90	0.720	0.490	1.26

¹ Neopeptone—skimmilk medium.

were selected. Strains 39aW-my and Mc-my were associated with *Candida krusei*. Methods of handling mother cultures described above were employed. Before the removal of samples for transfer to fresh media or for heat treatment, cultures were shaken to obtain a uniform mixture of film yeast and bacteria. The "mycoderm" was destroyed during the heat treatment; consequently, it did not interfere with plate counts or subsequent fermentation in the subcultures.

Results with *L. helveticus*, table 6, demonstrate that incubation for short consecutive periods at 37° C., and similar incubation periods followed by storage at 20° C. for 36 hours produced no differences in heat resistance between symbiotic and pure cultures. But when starters were incubated for 72 to 96 hours at 25° or 12-hour (37°) cultures were stored for 154 hours at 20°, the associated cultures were significantly more heat resistant than the pure cultures. It was observed that bacteria nearest the surface growth of the film yeast were far more active after heat treatment than bacteria farthest removed from the top of the mixed culture.

Similar treatment of *Str. thermophilus* also revealed that associated cul-

TABLE 6

Influence of incubation time and temperature of mother cultures of Lactobacillus helveticus (39aW) grown with a "mycoderm" (my) on the activity of subcultures at 37° C. following heat treatment at 62° C. for 30 minutes

Inoculating cultures			Heat-treated subcultures		
Type of mother culture	Incubation time and temperature	Titrat-able acidity	Plate count before heating	Plate count after heating	Drop in pH seven hours after heating
		per cent	millions/ml.	millions/ml.	
39aW	12 hrs. 37°	0.89	2.67	2.15	0.30
39aW-my	12 hrs. 37°	0.92	1.64	1.17	0.30
39aW	12 hrs. 37°	0.98	1.93	1.97	0.43
	36 hrs. 20°				
39aW-my	12 hrs. 37°	1.17	2.07	1.98	0.43
	36 hrs. 20°				
39aW	72 hrs. 25°	1.20	1.58	1.71	0.37
39aW-my	72 hrs. 25°	1.24	1.20	1.76	0.44
39aW	96 hrs. 25°	1.42	1.90	1.80	0.45
39aW-my	96 hrs. 25°	1.37	2.11	1.64	0.53
39aW	14 hrs. 37°	1.27	2.80	2.80	0.41
	154 hrs. 20°				
39aW-my	14 hrs. 37°				
	154 hrs. 20°				
* (a)		1.18	2.18	2.36	0.68
(b)		1.56	2.01	2.06	0.55
(c)		1.28	2.12	1.89	0.62

* (a) Subculture from topmost quarter of inoculating culture.

(b) Subculture from bottom quarter of inoculating culture.

(c) Subculture from inoculating culture previously thoroughly shaken to mix growth of top and bottom areas.

tures possessed greater activity after heating than pure cultures of this streptococcus. As shown in table 7 heat resistant mixed cultures were obtained when they were carried at 37° for 12 hours or at 25° for 72 hours. The influence of the "mycoderm" was not overcome by the number of successive transfers at 37° used here. In some instances increased activity may be attributable to higher numbers of survivors. It is also evident from data of table 7 that bacteria in the vicinity of "mycoderm" growth were more active after heat treatment than those farthest from the surface film.

The role played by *Candida krusei* resulting in enhanced heat resistance of these lactic starter bacteria will be discussed in another paper.

A summary of variations in time and temperature of incubation and storage which provided starter cultures of equivalent and maximum heat resistance appears in table 8.

DISCUSSION

The data support the belief that a variety of modifications can be applied to methods for handling starter cultures of thermoduric bacteria, depending

TABLE 7

Influence of incubation time and temperature of mother cultures of Streptococcus thermophilus (Mc) grown with a "mycoderm" (my) on the activity of subcultures at 37° C. following heat treatment at 64° C. for 30 minutes

Inoculating cultures			Heat-treated subcultures		
Type of mother culture	Incubation time and temperature	Titrat-able acidity	Plate count before heating	Plate count after heating	Drop in pH seven hours after heating
		per cent	millions/ml.	millions/ml.	
Mc	12 hrs. 37°	0.62	0.560	0.075	0.42
Mc-my	12 hrs. 37°	0.64	0.565	0.175	0.60
Mc	72 hrs. 25°	0.71	0.435	0.205	1.17
Mc-my	72 hrs. 25°	0.69	0.545	0.390	1.25
Mc	96 hrs. 25°	0.68	0.484	0.096	1.04
Mc-my	96 hrs. 25°	0.64	0.449	0.098	1.04
Mc	12 hrs. 37°	0.61	0.448	0.010	0.45
	36 hrs. 20°				
Mc-my	12 hrs. 37°	0.64	0.599	0.020	0.48
	36 hrs. 20°				
Mc	14 hrs. 37°	0.83	0.628	0.318	0.30
	154 hrs. 20°				
Mc-my	14 hrs. 37°				
	154 hrs. 20°				
*(a)		0.75	0.425	0.090	0.89
(b)		0.91	0.505	0.085	0.11
(c)		0.78	0.415	0.105	0.41

* (a) Subculture from topmost quarter of inoculating culture.

(b) Subculture from bottom quarter of inoculating culture.

(c) Subculture from inoculating culture previously thoroughly shaken to mix growth of top and bottom areas.

upon incubation temperature used, storage temperature available, and quality of the culture medium employed, without lessening the activity and heat resistance of the bacteria.

L. helveticus and *Str. thermophilus* may be grown in a good medium such as reconstituted skimmilk at a common temperature, 37°, to develop cultures of high heat resistance. If a better culture medium, one with added accessory substances is used, the heat resistance of cultures is not only assured but actually increased. This modification of methods for handling starter may be adopted when higher than usual cooking temperatures of curd are employed to avoid undesirable gas formation in Swiss cheese. The results of this investigation indicate that under such circumstances starter of great heat resistance can be produced by improving the culture medium, by incubation at 40° instead of 37° C., or by combining both procedures. In the case of *Str. thermophilus*, however, growth at 40° requires a medium of highest quality, and more careful control of incubation time is necessary to provide cultures of proper maturity. The results again emphasize the importance of a suitable and uniform starter culture medium, suggesting adoption of a definite plan of selection of high quality milk for this purpose.

TABLE 8

A summary of variations in time and temperature of incubation and storage which provided cultures of equivalent and maximum heat resistance

Starter	Incubation time and temperature	Quality of skim-milk medium
<i>L. helveticus</i> strain 39aW	$\left\{ \begin{array}{l} 12 \text{ hrs. } 37^{\circ} + 60 \text{ hrs. } 12^{\circ} \\ 24 \text{ hrs. } 37^{\circ} + 48 \text{ hrs. } 12^{\circ} \\ 10-15 \text{ hrs. } 40^{\circ} + 38 \text{ hrs. } 20^{\circ} \text{ or } 12^{\circ} \end{array} \right\}$	normal*
	$\left\{ \begin{array}{l} 12-24 \text{ hrs. } 37^{\circ} \text{ or } 40^{\circ} \\ 12 \text{ hrs. } 37^{\circ} + 36 \text{ hrs. } 12^{\circ} \\ 8-9 \text{ hrs. } 40^{\circ} + 40 \text{ hrs. } 12^{\circ} \text{ or } 4^{\circ} \\ 10-12 \text{ hrs. } 40^{\circ} + 12 \text{ hrs. } 12^{\circ} \\ 15 \text{ hrs. } 40^{\circ} + 9 \text{ hrs. } 4^{\circ} \end{array} \right\}$	fortified**
	$\left\{ \begin{array}{l} 12-24 \text{ hrs. } 37^{\circ} \\ 12 \text{ hrs. } 37^{\circ} + 84 \text{ hrs. } 20^{\circ} \\ 12 \text{ hrs. } 37^{\circ} + 36 \text{ hrs. } 12^{\circ} \end{array} \right\}$	normal*
<i>Str. thermophilus</i> strain C-3	$\left\{ \begin{array}{l} 12 \text{ hrs. } 37^{\circ} \text{ or } 40^{\circ} \\ 10-12 \text{ hrs. } 37^{\circ} + 84 \text{ hrs. } 12^{\circ} \\ 12 \text{ hrs. } 37^{\circ} + 36 \text{ hrs. } 4^{\circ} \\ 24 \text{ hrs. } 37^{\circ} + 24 \text{ hrs. } 12^{\circ} \\ 6-7 \text{ hrs. } 40^{\circ} + 42 \text{ hrs. } 12^{\circ} \text{ or } 4^{\circ} \end{array} \right\}$	fortified**

* Mother cultures grown in reconstituted skimmilk medium.

** Cultures carried in skimmilk medium with accessory substances added.

Results obtained with cultures transferred every 24 hours at either 37° or 40° C. suggest that starter cultures of *L. helveticus* and *Str. thermophilus* need not be transferred twice daily, as it is done in many cheese factories, to obtain heat resistant bacteria. The preparation of bulk starter for Swiss cheese need not be a daily task. With adequate incubation and storage facilities, bulk starter grown at 37° for 12 or 24 hours, for example, can be held from one to two days in a factory cold room. Thus enough starter can be prepared to supply the need for two days' manufacture. When cheese is made twice daily, starter for both lots can be prepared at the same time, a portion to be used in the morning and the remainder held in the cold room for use in cheese made in the afternoon.

The increased heat resistance of *L. helveticus* and *Str. thermophilus* grown in association with the film yeast, *Candida krusei*, revealed an aspect of symbiosis not recognized previously. Limited studies suggest the possibility that heat resistant cultures of these bacteria may be developed at temperatures considerably below the optimum for thermophilic bacteria, such as 30° C. It was demonstrated that bacteria near the "mycoderm" pellicle were more active after heating than bacteria from the same culture but at the lowest level, and that the beneficial influence of the film yeast extended for some distance below the surface of the culture. These results suggest that heat resistant cultures of these bacteria may be obtained by methods other than those customarily employed; such as, growing asso-

ciated cultures in thin layers of media, or in media with increased surface area.

Although the data have been discussed only in regard to their use by workers in the Swiss cheese industry, others who prepare cultures of thermophilic lactic acid bacteria required to grow rapidly after severe heat treatments may find helpful modifications of methods for handling such bacteria.

SUMMARY

1. Successive transfers every 12 and 24 hours at 37° and 40° C. resulted in more heat resistant cultures of *L. helveticus* at 40° than at 37° when mother cultures were grown in freshly reconstituted skim milk. In a medium with added accessory substances, such as Neopeptone or malt extract, 12 to 24 hours at 37° and 12 hours at 40° gave best results.

2. Consecutive transfers of *Str. thermophilus* at a constant temperature led to most active cultures when incubation in the fortified medium varied from 12 to 24 hours at 37°; but at 40° highest resistance to heat was shown by 12-hour cultures carried in the fortified medium.

3. Storage at 12° was least harmful to *L. helveticus* following incubation at 37°. Greatest heat resistance was observed when 12-hour cultures at 37° were held at 12° no longer than 60 hours, and 24-hour cultures at 37° could be stored for 48 hours at 12°. Improvement of the skim milk medium with Neopeptone increased slightly the heat resistance of stored mother cultures.

4. *Str. thermophilus* grown in the fortified medium at 37° for 10 to 12 hours and then stored 84 hours at 12° or 36 hours at 4°, and 24-hour cultures at 37° held 24 hours at 12° were equivalent in heat resistance to cultures transferred every 12 hours at 37°. Storage of cultures at 12° was better than at either 4° or 20° C.

5. After incubation at 40° for 8 to 15 hours, *L. helveticus* could be stored at 20°, 12°, or 4° C. until cultures were 48 hours old. Storage at 12° appeared to be least harmful to mother cultures.

6. When grown at 40° C. *Str. thermophilus* gave heat resistant cultures only when Neopeptone was added to the skim milk medium. Incubation at 40° for 6 to 7 hours followed by storage at either 12° or 4° C. until cultures were 48 hours old resulted in cultures as active after heating as those transferred serially at 37°.

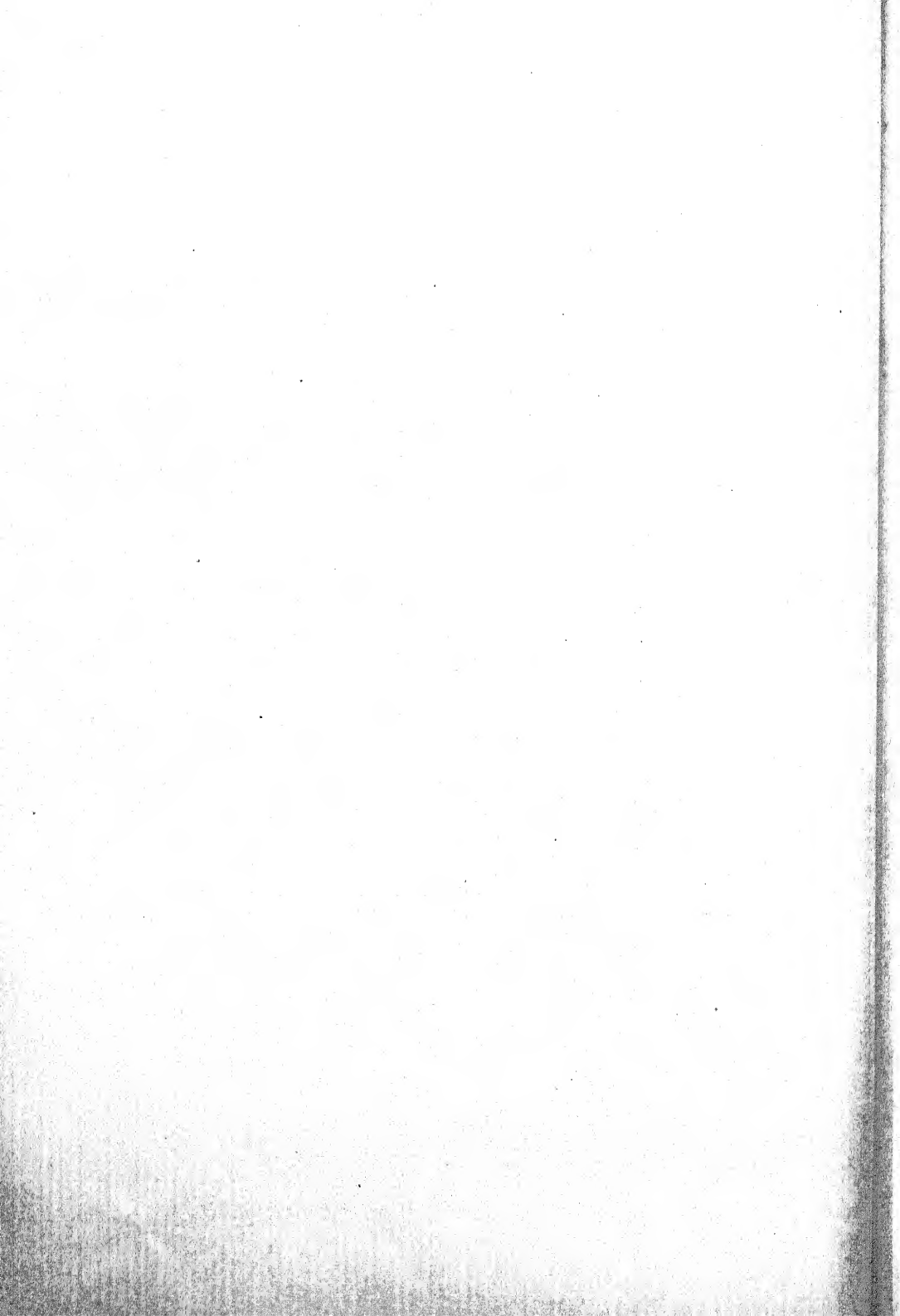
7. Mixed cultures of the film yeast, *Candida krusei*, and *L. helveticus* exhibited greater heat resistance than pure cultures of this bacterium after incubation at 25° C. for 72 to 96 hours, and also when incubation at 37° C. for 12 hours was followed by storage at 20° C. for 36 hours. Frequent transfer of associated cultures at 37° reduced the influence of the "myco-derm"; these cultures were equal in heat resistance to pure cultures of the bacteria carried under similar conditions.

8. *Str. thermophilus*, Mc, grown with "mycoderm" was more active after heating than pure cultures of the bacteria carried under similar conditions. Numerous successive 12-hour transfers of the associated culture at 37° did not decrease the influence of the film yeast.

9. In old cultures bacteria nearest the "mycoderm" film grew and fermented better following heat treatment than bacteria in areas farthest removed from the pellicle formed by the film yeast.

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THE LIMIT OF ERROR OF THE SIMPLIFIED VACUUM SOLIDS TEST AS APPLIED TO ICE CREAM MIX, EVAPORATED AND SWEETENED CONDENSED MILK

L. G. HARMON* AND K. M. RENNER

Department of Dairy Manufactures, Texas Technological College, Lubbock, Texas

INTRODUCTION

A Simplified Vacuum Solids Test for determining total solids in ice cream mix, condensed and evaporated milk has been developed by the Department of Dairy Manufactures of Texas Technological College. This method was devised in order to provide the dairy industry with a method for the estimation of total solids, which is accurate enough to meet the requirements of the commercial operator, rapid enough to be of commercial value, cheap enough to be available to all who wish to perform analysis for total solids, and simple enough to be performed by the average laboratory employee.

The purpose of this work was to determine the most satisfactory procedure for operating the Simplified Vacuum Solids Test; also to determine its accuracy as compared to the Official Method for determining total solids in evaporated milk, sweetened condensed milk, and to the adapted Official Method for ice cream mix.

The Simplified Vacuum Solids Test was originally designed in 1933 by Professor K. M. Renner, Head of the Department of Dairy Manufactures, Texas Technological College. Dr. William L. Ray, formerly of the Chemistry Department of Texas Technological College, offered valuable suggestions and advice on the construction of the original apparatus.

The first application of the test was made in the analysis for total solids in egg yolk. Numerous changes from the original design and procedure have been made in order to make the test more adaptable to liquid dairy products.

A number of commercial plants have adopted the Simplified Vacuum Solids Test. Until the present work was performed, there were no data available showing a statistical comparison of the Simplified Vacuum Solids Test to the Official Method.

REVIEW OF LITERATURE

The accuracy of several methods commonly used for testing condensed milk, evaporated milk and ice cream mix for total solids has been studied by previous investigators. Among the methods commonly used are the Mojonnier, Refractometric, and various adaptations of the Official.

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In studying the accuracy of methods for testing sweetened condensed milk, Fisher and Rice (2) compared results obtained by the Official method to the Mojonnier and a Modified Official method which they devised. These workers tested twenty samples of sweetened condensed milk by all three methods, and found that in all but one instance the Official method yielded the lowest results. The Mojonnier method gave the highest results on two-thirds of the tests. On these twenty samples the Mojonnier results averaged 0.57 per cent higher than the Official, whereas the Modified method averaged only 0.3022 per cent higher.

Fisher and Watts (3) made a study of some of the methods used in testing ice cream for total solids. They compared the adapted Official method to the Modified method recommended by Fisher and Rice (2) and to the Mojonnier method. They tested twelve samples of ice cream by each of the methods listed above. On all but two samples the adapted Official method yielded the lowest results, and the Mojonnier method the highest. The Mojonnier results averaged 0.44 per cent higher than the adapted Official, and the Modified method results averaged 0.23 per cent higher. These observations substantiated the results obtained by Fisher and Rice (2) in their work on sweetened condensed milk.

Rice and Miscall (6) in studying the Refractometric method of determining total solids in sweetened condensed milk, found that if accurate results are to be obtained the determinations must be made within a few hours after the batch is drawn because of the crystallizing tendency of lactose. These workers devised a formula by which total solids could be calculated from the refractive index, but they pointed out limitations of the reliability of the method in that the casein and albumen cannot be completely precipitated.

Menefee and Overman (4) also studied the Refractometric method of testing condensed and evaporated milk for total solids, using a copper sulphate solution to precipitate the protein. These investigators developed a formula for computing the total solids from the Refractometer reading of these products. They tested sixteen samples of condensed milk and found a standard error of estimate of 0.4641, with the greatest error on any sample 0.78. Twenty-five samples of evaporated milk were tested, and the results showed a standard error of estimate of 0.49, with the largest single error being 0.97.

LABORATORY EQUIPMENT

The following laboratory equipment is required for assembling the Simplified Vacuum Solids Testing Device:

- (1) 1—1000-ml. Pyrex Beaker
- (2) 1—1000-ml. Suction Flask
- (3) Pyrex Test Tubes 7" long and 1" in diameter

ing and testing, the samples were kept at a temperature of about 40° F. to minimize bacterial action and acid development. No sample was held longer than two days, that being the length of time required to perform the series of fifty tests which were performed on each sample.

PREPARATION OF SAMPLES

None of the sweetened condensed milk tested in this work had been homogenized, whereas all of the ice cream mix and evaporated milk samples had been homogenized. The sweetened condensed skim milk samples were prepared according to the recommendations of the Official method (1) for total solids determination. An attempt was made to prepare the sweetened condensed whole milk samples in the same manner, but was unsuccessful because the butterfat churned when the warm diluted sample was agitated. The sweetened condensed whole milk samples were maintained at a temperature of about 50° F. while being diluted with an equal weight of cold distilled water, stirred and mixed. The homogenized ice cream mix and evaporated milk samples required no preliminary treatment other than mixing. All samples were thoroughly mixed by stirring and pouring several times before weighing each sample.

TESTING PROCEDURE

A large (1"×7") test tube is placed on the scale pan of an analytical balance, and is held in an upright position by a wire clasp designed for the purpose. One to two grams of the sample is weighed into the test tube. The test tube containing the sample is then placed in the 1000-ml. beaker which contains boiling distilled water. The test tube and beaker are supported by a ring stand as shown in the illustration. The two-hole rubber stopper, into which is inserted the thistle tube and suction line, is seated in the test tube, and the water then turned into the aspirator.

As the vacuum is created in the test tube, the sample boils vigorously and spreads in a thin film over the interior of the test tube. Care must be exercised at this point to prevent any of the sample from escaping into the suction line. This is controlled by using proper volume and dilution of sample, and if necessary temporarily breaking the vacuum. The sample is thus heated for fifteen minutes, then removed from the boiling water, dried thoroughly, cooled in a desiccator and weighed. The per cent total solids is calculated by dividing the weight of residue by the weight of sample and multiplying by 100. A proper correction must be made according to the amount of the original dilution.

EXPERIMENTAL

A large number of preliminary analyses was performed in an attempt to improve on the procedure previously recommended by Renner (5) in

which the weighings were performed on a Torsion balance. In the operation of the Simplified Vacuum Solids Test, the best results were obtained when a sample weighing from one to two grams was used, diluted in such a manner as to contain between 18 per cent and 35 per cent total solids, heated at 212° F. under at least a 24-inch vacuum for fifteen minutes, and cooled in a desiccator before weighing.

In order to determine the accuracy of the Simplified Vacuum Solids Test, groups of fifty tests for total solids were performed on each of the following products: Evaporated Milk, Ice Cream Mix, Sweetened Condensed Whole Milk and Sweetened Condensed Skim Milk.

Total solids determinations were also made by the Official method (1) or in the case of ice cream mix, an adapted Official method (2). The error of the Simplified Vacuum Solids method was measured from the average of four Official results on each sample.

For the purpose of measuring the accuracy of the results, the following statistical methods were applied to all groups of tests:

1. The arithmetic mean of error was determined on the error and on the total solids.
2. The standard deviation was determined on the error and on the total solids.
3. The coefficient of variation was determined on the total solids.

The accuracy of the Simplified Vacuum Solids Test as compared to the Official method for determining the total solids in evaporated milk, ice cream mix, sweetened condensed whole milk and sweetened condensed skim milk is shown in tables 1, 2, 3 and 4, respectively.

DISCUSSION AND CONCLUSIONS

One of the greatest difficulties in the operation of the Simplified Vacuum Solids Test is the prevention of the loss of small particles of sample into the suction line. This difficulty can be controlled by using a small sample (one or two grams) which has been diluted so as to contain between 18 per cent and 35 per cent total solids. Low solids samples have more tendency to be drawn into the suction line, whereas high solids samples having a high viscosity do not spread in a thin uniform film over the interior of the surface of the test tube, and thus have less evaporation surface.

The arithmetic mean of the error and of the total solids was determined in order to show the simple average of the error. The standard deviation was used in comparing the error of the results, because it is a measure of absolute variability. The coefficient of variability was also calculated, since it shows the ratio of the standard deviation to the arithmetic mean.

In estimating the total solids in evaporated milk, the Simplified Vacuum Solids method compared favorably with other methods in use, in that the arithmetic mean of error was 0.1529.

TABLE 1
Evaporated milk
 Official T. S. = 26.57%

Sample No.	Wt. of sample	Wt. of residue	% T. S. determined	Error
1	2.0539	.5487	26.72	.15
2	2.0137	.5369	26.66	.09
3	2.0145	.5380	26.71	.14
4	1.9976	.5321	26.64	.07
5	2.0113	.5356	26.62	.05
6	2.0065	.5340	26.61	.04
7	1.9418	.5200	26.77	.20
8	1.9404	.5190	26.74	.17
9	1.8876	.5035	26.67	.10
10	1.8689	.4989	26.69	.12
11	1.8749	.5017	26.75	.18
12	1.8705	.5005	26.75	.18
13	1.8896	.5058	26.74	.17
14	1.9391	.5193	26.78	.21
15	2.0827	.5570	26.74	.17
16	2.1160	.5654	26.72	.15
17	2.0820	.5548	26.64	.07
18	2.0396	.5462	26.78	.21
19	2.0342	.5443	26.76	.19
20	2.0576	.5506	26.76	.19
21	1.9757	.5277	26.71	.14
22	2.0303	.5412	26.66	.09
23	1.9553	.5251	26.85	.28
24	2.0012	.5347	26.72	.15
25	1.9779	.5290	26.75	.18
26	1.9701	.5278	26.78	.21
27	1.9333	.5176	26.77	.20
28	1.9750	.5294	26.78	.21
29	1.9638	.5259	26.78	.21
30	2.1213	.5647	26.62	.05
31	2.0613	.5500	26.68	.11
32	2.0344	.5427	26.68	.11
33	2.0046	.5333	26.60	.03
34	2.0279	.5412	26.69	.12
35	1.9319	.5172	26.77	.20
36	1.9926	.5334	26.77	.20
37	1.9467	.5208	26.75	.18
38	1.9563	.5237	26.76	.19
39	1.9365	.5090	26.75	.18
40	1.8891	.5058	26.77	.20
41	1.9449	.5202	26.75	.18
42	1.9555	.5231	26.75	.18
43	1.9386	.5181	26.73	.16
44	1.9120	.5128	26.82	.25
45	1.9318	.5171	26.76	.19
46	1.8735	.5030	26.84	.27
47	2.1404	.5690	26.58	.01
48	2.0434	.5456	26.70	.13
49	2.0250	.5386	26.60	.03
50	2.1125	.5655	26.72	.15

Statistical analysis of results in table 1

1. The arithmetic mean of error = 0.1529.
2. The standard deviation of the error = 0.0624.
3. The arithmetic mean of the total solids = 26.72.
4. The standard deviation of the total solids = 0.0624.
5. The coefficient of variation of the total solids = 0.2335.
6. No minus errors were obtained.
7. The range of error was from 0.01 to 0.28.
8. Ninety-six per cent of the results were within 0.25 per cent of the Official.

TABLE 2
Ice cream mix
Official T. S. = 36.87%

Sample No.	Wt. of sample	Wt. of residue	% T. S. determined	Error
1	1.1645	.4360	37.44	.57
2	1.0222	.3801	37.18	.31
3	1.0284	.4041	37.33	.46
4	1.0298	.3908	37.94	1.07
5	1.0496	.3900	37.16	.29
6	1.0554	.3936	37.29	.42
7	1.1056	.4110	37.18	.31
8	1.0408	.3863	37.12	.25
9	1.0026	.3732	37.22	.35
10	1.0212	.3818	37.39	.52
11	1.0438	.3894	37.31	.44
12	1.0196	.3823	37.49	.62
13	1.0076	.4764	37.36	.49
14	1.0107	.3787	37.47	.60
15	1.0130	.3755	37.07	.20
16	1.0032	.3779	37.67	.80
17	.9979	.3740	37.48	.61
18	.9899	.3657	36.94	.07
19	1.0109	.3759	37.14	.27
20	1.0651	.3965	37.29	.42
21	1.0214	.3819	37.39	.52
22	1.0567	.3950	37.38	.51
23	1.0170	.3799	37.35	.48
24	1.0442	.3889	37.24	.37
25	1.0653	.3993	37.48	.61
26	1.0620	.3936	37.07	.20
27	.9936	.3733	37.57	.70
28	1.0250	.3855	37.71	.84
29	1.0198	.3823	37.48	.61
30	.9857	.3681	37.45	.58
31	.9973	.3725	37.36	.49
32	1.0210	.3815	37.36	.49
33	1.0301	.3887	37.73	.86
34	1.0252	.3830	37.41	.54
35	1.0221	.3804	37.21	.34
36	1.0132	.3811	37.61	.74
37	1.0060	.3788	37.65	.78
38	.9908	.3695	37.29	.42
39	.9901	.3719	37.56	.69
40	.9717	.3649	37.55	.68
41	1.0038	.3742	37.28	.41
42	.9723	.3639	37.43	.56
43	.9643	.3590	37.23	.36
44	1.0105	.3790	37.49	.62
45	.9943	.3675	36.96	.09
46	.9673	.3607	37.29	.42
47	.9504	.3589	37.76	.89
48	.9994	.3755	37.57	.70
49	1.0026	.3762	37.52	.65
50	.9647	.3580	37.11	.24

Statistical analysis of results in table 2

1. The arithmetic mean of the error = 0.5090.
2. The standard deviation of the error = 0.1521.
3. The arithmetic mean of the total solids = 37.38.
4. The standard deviation of the total solids = 0.2261.
5. The coefficient of variation of the total solids = 0.6032.
6. No minus errors occurred in the results.
7. The range of error was from 0.07 to 1.07.
8. Twelve per cent of the results were within 0.25 per cent of the adapted Official.
9. Fifty per cent of the results were within 0.50 per cent of the adapted Official.
10. Ninety-eight per cent of the results were within 1 per cent of the adapted Official.
11. Only one sample had an error in excess of 1 per cent.

TABLE 3
Sweetened condensed whole milk
 Official T. S. = 70.86%

Sample No.	Wt. of sample	Wt. of residue	% T. S. determined	Error
1	1.1106	.3961	71.34	.48
2	1.1004	.3918	71.22	.36
3	1.0915	.3905	71.56	.70
4	1.1130	.3966	71.26	.40
5	1.0959	.3906	71.28	.42
6	1.0982	.3909	71.18	.32
7	1.0640	.3795	71.34	.48
8	1.0743	.3815	71.02	.16
9	1.0614	.3772	71.08	.22
10	1.0601	.3780	71.32	.46
11	1.0776	.3834	71.16	.30
12	1.0439	.3721	71.30	.44
13	1.0458	.3732	71.36	.50
14	1.1027	.3942	71.48	.62
15	1.0841	.3875	71.48	.62
16	1.0618	.3781	71.20	.34
17	1.0854	.3880	71.50	.64
18	1.1314	.4017	71.00	.14
19	1.0372	.3700	71.34	.48
20	1.0340	.3705	71.66	.80
21	1.0289	.3664	71.22	.36
22	1.0313	.3662	71.02	.16
23	1.0531	.3784	71.86	1.00
24	1.0423	.3693	70.86	.00
25	1.0397	.3682	70.82	-.04
26	1.0332	.3704	71.70	.84
27	1.0489	.3726	71.04	.18
28	1.0286	.3667	71.30	.44
29	1.0418	.3720	71.42	.56
30	1.0392	.3691	71.04	.18
31	1.0503	.3760	71.58	.72
32	1.0377	.3672	70.78	-.08
33	1.0645	.3775	70.92	.06
34	1.0320	.3680	71.32	.46
35	1.0468	.3724	71.16	.30
36	1.0268	.3649	71.08	.22
37	1.0595	.3773	71.22	.36
38	1.0574	.3768	71.26	.40
39	1.0571	.3758	71.12	.26
40	1.0462	.3718	71.08	.22
41	1.0325	.3683	71.34	.48
42	1.0363	.3695	71.32	.46
43	1.0411	.3778	71.42	.56
44	1.0391	.3701	71.24	.38
45	1.0494	.3747	71.42	.56
46	1.0314	.3673	71.22	.36
47	1.0940	.3910	71.48	.62
48	1.0637	.3779	71.06	.20
49	1.0500	.3727	71.00	.14
50	1.0211	.3630	71.10	.24

Statistical analysis of results in table 3

1. The arithmetic mean of the error = 0.3944.
2. The standard deviation of the error = 0.2284.
3. The arithmetic mean of the total solids = 71.25.
4. The standard deviation of the total solids = 0.2278.
5. The coefficient of variation of the total solids = 0.3197.
6. Four per cent of the results had a minus error.
7. The range of error of the results was from minus 0.08 to plus 1.00.
8. Thirty per cent of the results were within 0.25 per cent of the Official.
9. Seventy-six per cent of the results were within 0.50 per cent of the Official.
10. All of the results were within 1 per cent of the Official.

TABLE 4
Sweetened condensed skim milk
 Official T. S. = 70.96%

Sample No.	Wt. of sample	Wt. of residue	% T. S. determined	Error
1	1.0265	.3693	71.96	1.00
2	1.0176	.3697	72.66	1.70
3	1.0274	.3695	71.92	.96
4	1.0227	.3665	71.70	.74
5	1.0384	.3702	71.70	.74
6	1.0245	.3683	71.90	.94
7	1.0292	.3700	71.90	.94
8	1.0310	.3704	71.86	.90
9	1.0202	.3676	72.06	1.10
10	1.0263	.3711	72.32	1.36
11	1.0299	.3699	71.84	.88
12	1.0359	.3741	72.22	1.26
13	1.0320	.3750	72.66	1.70
14	1.0221	.3680	72.00	1.04
15	1.0118	.3641	71.98	1.02
16	1.0327	.3728	72.20	1.24
17	1.0477	.3799	73.28	2.32
18	1.0335	.3733	72.24	1.28
19	1.0473	.3805	72.66	1.70
20	1.0224	.3766	71.74	.78
21	1.0535	.3778	71.72	.76
22	.9979	.3583	71.82	.86
23	.9965	.3569	71.64	.68
24	1.0570	.3787	71.66	.70
25	1.0377	.3731	71.92	.96
26	1.0225	.3650	71.40	.44
27	1.0386	.3715	71.54	.58
28	1.0663	.3859	72.38	1.42
29	1.0353	.3720	71.86	.90
30	1.0385	.3705	71.36	.40
31	1.0062	.3649	72.64	1.68
32	1.0573	.3813	72.12	1.16
33	1.1344	.4026	70.98	.02
34	1.0102	.3681	72.88	1.92
35	1.0207	.3706	72.58	1.62
36	1.0266	.3681	71.72	.76
37	1.0291	.3717	72.24	1.28
38	1.0253	.3745	73.06	2.10
39	1.0352	.3744	72.34	1.38
40	1.0134	.3702	73.06	2.10
41	1.0307	.3725	72.28	1.32
42	1.0296	.3732	72.50	1.54
43	1.0214	.3679	72.04	1.08
44	1.0418	.3760	72.18	1.22
45	1.0418	.3787	72.70	1.74
46	1.0394	.3813	73.40	2.44
47	1.0333	.3893	75.36	4.40
48	1.0335	.3828	74.08	3.12
49	1.0269	.3706	72.18	1.22
50	1.0294	.3677	71.44	.48

Statistical analysis of results in table 4

1. The arithmetic mean of the error = 1.2776.
2. The standard deviation of the error = 0.7214.
3. The arithmetic mean of the total solids = 72.24.
4. The standard deviation of the total solids = 0.7220.
5. The coefficient of the variation of the total solids = 0.9996.
6. No minus errors occurred in the results.
7. The range of error of the results was from 0.02 to 4.40.
8. Only one result, or 2 per cent, was within 0.25 per cent of the Official.
9. Eight per cent of the results were within 0.50 per cent of the Official.
10. Forty-two per cent of the results were within 1 per cent of the Official.
11. Fifty-eight per cent of the results varied more than 1 per cent from the Official.

Menefee and Overman (4) applied the Refractometric method to evaporated milk, and reported a standard error of estimate of 0.49.

The Simplified Vacuum Solids Test applied to ice cream mix yielded an arithmetic mean of error of 0.5090. Fisher and Watts (3) compared the Mojonnier method to the Official, and reported an average variation on the Mojonnier of 0.4830.

Sweetened condensed whole milk samples tested by the Simplified Vacuum Solids method showed an arithmetic mean of error of 0.3944. Fisher and Watts (3) tested sweetened condensed whole milk by the Mojonnier method, and reported an arithmetic mean of error of 0.57, and Menefee and Overman (4) using the Refractometric method reported an arithmetic mean of error of 0.4641.

The Simplified Vacuum Solids method results were less satisfactory on sweetened condensed skim milk than on the other products. The arithmetic mean of error was 1.2776. The Mojonnier and Refractometric methods can be applied to sweetened condensed skim milk and sweetened condensed whole milk with about equal accuracy.

The results obtained by the Simplified Vacuum Solids method compared favorably with results obtained from other methods by previous workers when the comparisons were made by statistical devices commonly used for measuring accuracy.

The Simplified Vacuum Solids Test can be performed in twenty to twenty-five minutes, which compares favorably with the time required for other common total solids tests.

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EVIDENCE FOR THE PRESENCE OF SMOOTH MUSCLE ELEMENTS SURROUNDING THE ALVEOLI OF THE MAMMARY GLAND*

ERIC W. SWANSON AND C. W. TURNER

Department of Dairy Husbandry, University of Missouri, Columbia, Missouri

In connection with the renewed interest concerning the relation of the nerves, hormones and muscles which coordinate the important process of milk "ejection" or "letting down" at the time of milking, it seemed of interest to further investigate the type of cells surrounding the alveoli.

The literature on the subject is reviewed by Turner (1). The earlier investigators quite generally held that muscular cells or epithelial cells with contractile properties (myo-epithelial cells) were present in the subepithelial area of the alveolus. Recent observers have expressed doubt that these cells surround the alveolus directly but have suggested that the smooth muscle or myo-epithelial cells observed surround the capillaries instead.

For many years it has been known that extracts of the posterior lobe of the pituitary called "pituitrin," when injected into lactating animals cause an apparent contraction of the mammary gland and milk becomes available which previously could not be removed. (For review see Turner & Slaughter (2).) As pituitrin is considered to cause contraction of smooth muscles, especially of the uterus and blood vascular system, it seemed logical to assume that the effect of pituitrin upon the mammary gland was to produce a contraction of the muscular elements, forcing down the milk accumulated in the lumina of the alveoli and storage spaces of the duct system.

While the effect of pituitrin upon the mammary gland, uterus and vascular system has been known for a long time, only recently has evidence begun to appear indicating that it may play a role in the contraction of the udder musculature and the "letting down" of milk following the stimulation of the teats at milking time (3, 4, 5). The present concept of the relation of the nerves, posterior pituitary, adrenal and udder has been outlined (6). It should be understood, however, that positive proof is still lacking. As several phases of the problem are being investigated in our laboratory, it seemed desirable to re-examine the cellular structures surrounding the alveoli in order to determine whether muscle cells are present to aid in milk removal.

Experimental technique. The udder of a cow in advanced lactation was obtained. Blocks of tissue were placed in Bouin's fluid within an hour post mortem. The usual procedure of sectioning tissue was followed. Several

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stains were tried but the best results were obtained when the sections were stained first in Delafield's hematoxylin for 5 minutes, washed in water, stained 30 seconds in Van Giesen's stain, washed in water again, then passed rapidly up the graded alcohols ($\frac{1}{2}$ min. in each) cleared and mounted with clarite.

Observations. Van Giesen's stain very clearly differentiated the connective tissue by a brilliant red color. The epithelial and muscle cells were stained yellow to brown with well defined purplish brown nuclei. The smooth muscle cells were differentiated from the epithelial cells by the shape of the nuclei, the former being narrow, oblong structures while the latter were slightly ovoid or round. When the cell walls were observed, the epithelial cells were square or rectangular in shape while the muscle cells were long, narrow and more or less pointed at their ends.

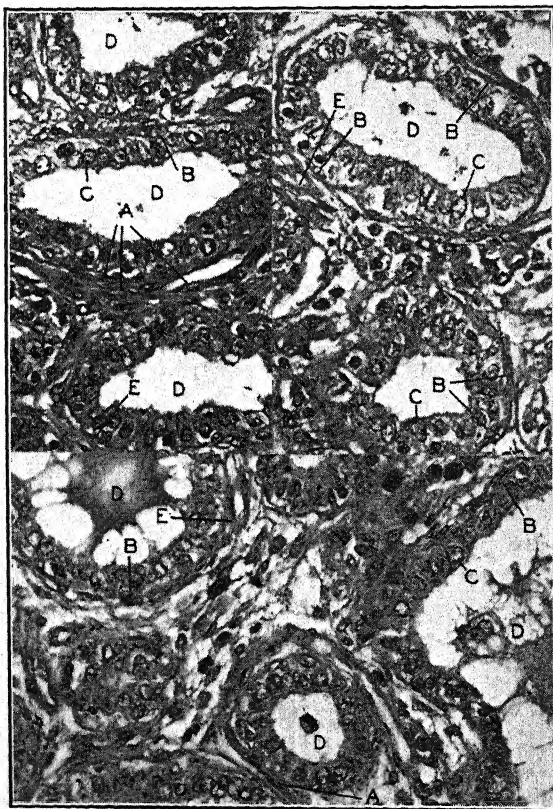


FIG. 1. Sections of mammary gland tissue from a cow in advanced lactation showing cell types of and around the alveoli. A, smooth muscle cells in the interlobular connective tissue; B, smooth muscle cells adjacent to the secretory epithelium; C, secretory epithelial cells; D, lumen of the alveolus; E, interlobular connective tissue fibers. $\times 375$.

Occasional bundles of yellow smooth muscle cells were found scattered among the red connective tissue fibers. Individual isolated smooth muscle cells were also noticed in the interlobular connective tissue. The subalveolar cells were carefully examined. Smooth muscle cells were identified around nearly every alveolus examined (Fig. 1). At times these cells were observed in the interalveolar connective tissue, but frequently they appeared between the connective tissue membrane and the secretory epithelial cells. In no case were the smooth muscle cells observed to form a continuous band around the alveolus. Rather, they seemed to be isolated from each other and were probably of the basket cell type observed by Lenfers (7) and others.

It is difficult to determine definitely whether or not the smooth muscle cells observed directly around the alveolar epithelium are or are not associated with capillaries. However, the capillaries furnishing blood to the secretory epithelium would be of the finest calibre. As a general rule there are neither muscle fibers nor connective tissue in the walls of the true capillaries. It would appear reasonable to believe that these isolated muscle cells around the alveoli contract, thus increasing the pressure on the alveolar lumen contents and forcing the milk into the duct system. The smooth muscles around the ducts seemed to be more numerous and more distinct than those around the alveoli. Upon their contraction there would undoubtedly be a tendency to reduce the length and diameter of the ducts to aid the forward movement of the milk. While no tendency was observed for the muscle cells of the ducts to form in a circular ring or sphincter and it is not believed that muscular sphincters are present at the branching of the ducts, the present method of examination of the tissue does not entirely disprove their presence.

DISCUSSION

The commonplace act of milking sets in motion a complex series of physiological events which are just beginning to be appreciated. The removal of milk from a dairy cow is not as simple as turning a spigot on a barrel and letting the milk flow out. It is believed that the stimulus from the teats, or other sensory stimuli associated with milking, transmits to the pituitary (posterior lobe) a nerve impulse which causes a discharge of pituitrin into the blood stream. Upon reaching the udder, pituitrin causes the contraction of the muscle fibers surrounding the alveoli and ducts and forces the milk into the larger collecting spaces and cistern toward the teat. There is thus an inward squeeze upon every part of the gland which results in aiding the milker to get a greater part of the milk present.

The present study is believed to show that there are muscle cells surrounding the alveoli which upon contraction would aid in evacuating the milk from the lumen, and further that muscle cells line the fine as well as the coarse milk ducts, which upon contraction propels the milk forward to the

teat. Sphincter muscles around the milk ducts were not observed and their presence seems illogical if the above explanation of "letting down" milk is correct. The presence of duct sphincters would call for a complicated control system whereby certain smooth muscles of the ducts would be contracted while the sphincter at the same time must necessarily be relaxed.

SUMMARY

An examination of the udder of a lactating cow by histological means and suitable staining showed the presence of cells beneath the secretory epithelium of the alveoli and in the interlobular spaces which had the appearance and staining properties of smooth muscle fibers. These cells were not observed to form a continuous band around the individual alveoli but were spaced at intervals below the epithelial cells. They are believed to surround the alveoli and to aid in the expulsion of milk from the lumen and not to be associated with blood capillaries. The walls of the duct system also contained similar smooth muscle cells but no tendency was observed for these cells to form muscular sphincters which would, upon contraction, impede the flow of milk.

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THE RELATIONSHIP OF FAT TO QUALITY, AND METHODS OF STANDARDIZING THE FAT CONTENT, IN SWISS CHEESE

GEORGE P. SANDERS, ROBERT R. FARRAR, FRED FEUTZ, AND
ROBERT E. HARDELL¹

*Division of Dairy Research Laboratories, Bureau of Dairy Industry,
U. S. Department of Agriculture²*

As a result of experimental research conducted in these laboratories and data obtained in factories in the Swiss cheese-producing areas in Wisconsin, Ohio, Idaho, and Pennsylvania, much information has been collected with respect to causes of variations in quality of Swiss cheese. It was shown, in results published previously (3), that the use of milk of poor bacteriological quality (methylene blue reduction time less than 3 hours) resulted in a decrease in the average grade of the cheese, and also that a complete lack of ripeness in the milk was apparently detrimental. In a recent report (4), methods were described which can be used to control the amount of moisture in the green cheese, and it was demonstrated that methods used to reduce the percentage of moisture resulted in a general improvement in quality of cheese.

This paper deals with the relationship between the percentage of fat in dry matter in the cheese and its quality, and with procedures used in predicting the percentage of fat in dry matter in the cured cheese.

RELATIONSHIP OF PERCENTAGE OF FAT IN DRY MATTER TO QUALITY

Factory cheese. The relationship of percentage of fat in dry matter to quality, for 844 cured cheeses in 39 factories, is shown in figure 1. It was found that the proportion of good cheese was largest among those which contained between 45 and 46 per cent fat in dry matter. The results show also that the average quality was better in cheese containing more than 48 per cent fat in dry matter than in cheese containing less than 43 per cent.

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² The work on factory cheese described herein was conducted with the cooperation of the Departments of Dairy Industry of the University of Wisconsin and the Ohio State University.

This was true for data from 19 factories located in Wisconsin, and likewise from 18 factories located in Ohio.

The grades of some of the high-fat cheeses were reduced because the cheeses were soft, weak, or pasty in body, and because they contained "glass" or splits in the curd (glaesler defect). A large proportion of the D grade (grinder) cheeses, in several factories, were soft or weak in body. Results of recent experiments in the laboratory indicate that extreme softness or weakness of body may be a result of the use of milk that contains an abnormally low proportion of casein to other non-fatty solids, and that the presence of a high proportion of fat tends to accentuate the softness.

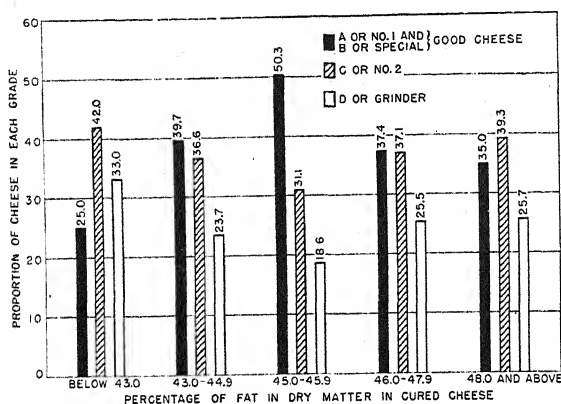


FIG. 1. Relation of percentage of fat in dry matter to quality (844 factory cheeses).

Laboratory cheese. Results were slightly different in the case of smaller, experimental cheese made in the laboratory plant, where the milk used is relatively high in percentage of total solids, and in curd tension, as compared with that used in the cheese-producing areas. Data for 30 pairs of experimental cheese are shown in table 1. These cheeses were the same ones for which moisture and yield data were quoted and discussed in connection with the results shown under variation No. 5 in table 3 of a former publication (4). For one cheese of each pair, the percentage of fat in the milk was reduced below that in the milk for the other by adding a relatively larger quantity of skim milk. Most of these cheeses—particularly those of low fat content—were too firm in body. This property seems to be characteristic of cheese made from high-solids, firm-curd milk, and is overcome to some extent by the incorporation of relatively more fat. The highest quality in factory cheese, on the other hand, was usually found among those which contained less fat in dry matter than was present in the best laboratory cheese.

In the experiments for which data are shown in table 1, better quality was secured in cheese containing more than 48 per cent fat in dry matter than in cheese containing less than 45 per cent. The scores of the low-fat

TABLE 1

Data showing relationship of percentage of fat in Swiss cheese kettle milk to composition and quality of experimental cheese (averages for 30 pairs of 60-lb. cheese, cured 2-2/3 months)

Milk	Cured cheese					
Fat	Fat in dry matter	Total score	Decrease in score			
			Overset	Texture too firm	Glaesler defect (cracks in curd)	Flavor defects
%	%	points	points	points	points	points
2.9	44.12	69.4	9.4	4.5	0.3	4.6
3.4	48.60	75.0	6.2	1.3	2.6	3.1

cheeses were reduced more than those of the high-fat ones for being overset, for defects in flavor, and for excessively firm body. On the other hand, a few of the high-fat cheeses contained the glaesler or curd-splitting defect, but there was less evidence of it in the low-fat ones. The low-fat cheeses rose more rapidly, and to a greater extent, than the high-fat ones. When cured, they contained an average of 0.55 per cent more moisture than the high-fat ones. Kettle whey from the low-fat cheeses contained an average of 7.23 per cent total solids and 0.50 per cent fat; corresponding data for whey from the high-fat ones were 7.44 and 0.67, respectively.

PREDICTION OF PERCENTAGE OF FAT IN DRY MATTER

Procedures based on analyses of samples of kettle curd. Because of damage done to uncured cheese by taking plug samples, it is very desirable to have a reasonably accurate analytical method for securing and testing samples of kettle curd rather than of uncured or green cheese. Having data on composition of curd, and on its relationship to composition of cured cheese, the cheesemaker can alter the percentage of fat in the milk on the basis of results of curd analyses, and possibly alter the making process, in order to regulate the proportion of fat in cheese made on successive days.

Securing and pressing samples of kettle curd. A photograph of equipment used in securing and preparing samples of kettle curd is shown in figure 2. Two samples of kettle contents were taken immediately before dipping from a point several inches behind the brake where the curd is well mixed by the brake and where the larger particles rise to the surface in greatest numbers. Each sample was taken with a dipper having a bronze screen bottom made of 18-mesh, 28-gauge wire. The cup was 2½ inches in height and 2½ inches in diameter, and the length, including handle, 14 inches. Two dippers were inserted in the revolving kettle contents to a depth corresponding to the length of the handle and were withdrawn with curd contents. Whey was allowed to drain for 2 minutes, after which the curd was loosened from the sides and bottoms by tapping the dippers and curd from both dippers was then placed in one of the metal pressure cylinders. The reason for

taking two small samples instead of one large one was that the probability of securing a representative sample was thus increased.

The cylinders used for pressing the curd samples were $1\frac{1}{8}$ inch in internal diameter and $3\frac{1}{2}$ inches in height. The bottom consisted of a flat metal plate containing about 200 holes each 0.05 inch in diameter, spaced about $\frac{1}{8}$ inch apart. The construction was such that whey drained freely without curd being squeezed out. Each cylinder had 4 legs, each $\frac{1}{4}$ inch long, and was provided with a metal plunger about $3\frac{1}{16}$ inches long, of the proper diameter to fit snugly within the cylinder without binding, and weighing 1000 grams.

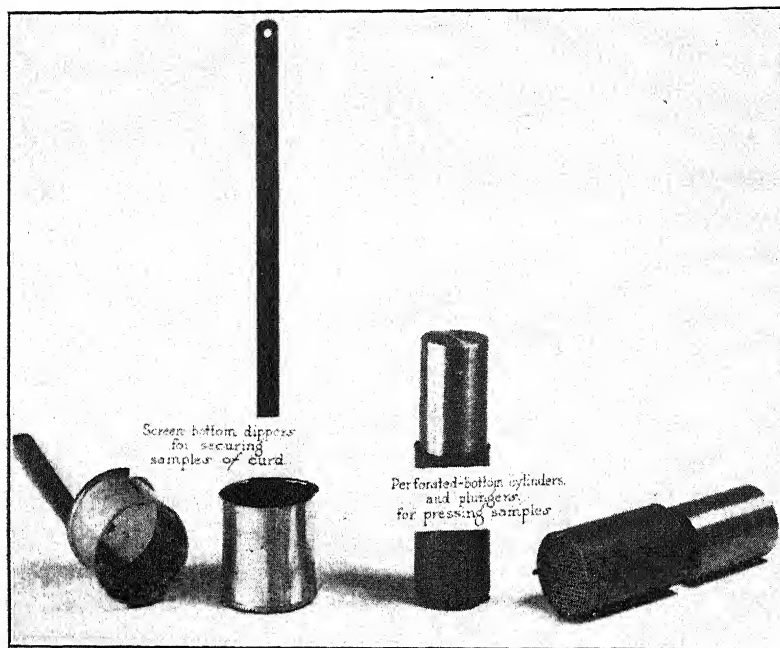


FIG. 2. Equipment for securing and preparing samples of curd from Swiss cheese kettle for analyses.

The plunger was placed upon the sample in the cylinder immediately and allowed to press for 30 minutes. The cylinder was inverted occasionally to allow whey to drain from the upper surface of the sample. The curd, when removed from the cylinder, was either analyzed at once or was wrapped tightly in tin foil, placed in a small, air-tight container, and kept in a refrigerator until analyzed.

For analyses of moisture and fat, each pressed curd sample was sliced vertically through the center, the outer portions were cut off and discarded, and thin slices were removed rapidly from the flat surface. Moisture samples were prepared by cutting the slices rapidly into small pieces with a sharp spatula in a small jar or tumbler; fat samples, by cutting the freshly-prepared slices into strips.

The equipment described above was designed and used in such a way that all curd samples were secured and prepared mechanically in a uniform manner, each one yielding a solid, compact mass which was comparatively similar to the cheese itself in consistency and in composition.

A valuable and exhaustive study of the control of composition in Swiss cheese has been made by Price (2) and his associates. They analyzed a large number of samples of kettle curd and of the corresponding cured cheese and found that the accuracy of prediction of fat in dry matter of cured cheese, based on values obtained in curd analyses, was limited to a range of 1 per cent in 66 per cent of the instances and 2 per cent in 95 per cent of the instances. In their method, referred to herein as the older method, samples of curd were taken from the kettle with a dipper, squeezed by hand to remove as much as possible of the whey, and then analyzed while in a crumbly condition.

Relative accuracy of predictions based on curd analyses. The accuracy of results obtained by the new, pressed-curd method was compared with the accuracy of results obtained by the older, manually-prepared curd method. Percentages of fat in dry matter found by analyzing samples of the cured cheese were taken as the actual or correct values; the amount by which the fat-in-dry-matter value of each sample of curd differed from that of the corresponding sample of cured cheese was calculated. The variations were grouped, according to size, and yielded the data shown in table 2. The results indicate that the use of the new method yielded a greater degree of precision than the use of the older one. For the new method, the average deviation of fat-in-dry-matter values of curd compared with factory-cured cheese was 0.82 per cent, and the standard deviation 1.02 per cent. For the older method, corresponding values were: Average deviation, 1.30 per cent; standard deviation, 1.69 per cent.

TABLE 2

Data showing relative accuracy of results of two different curd-analysis methods of estimating the percentage of fat in dry matter in Swiss cheese

Method of sampling	Samples		Percentage of predicted values falling within specified limits of variation from actual values found in cured cheese				
	Number	Source	0 ± 0.5	0 ± 1.0	0 ± 1.5	0 ± 2.0	0 ± 2.5
1	49	Laboratory	% 49.0	% 87.8	% 100.0	%	%
	458	Factory	41.1	69.4	87.5	95.8	98.2
2	136	Factory	32.4	52.2	67.6	80.1	89.0

Method 1 (new method)—Analyses of mechanically-pressed samples of kettle curd.

Method 2 (old method)—Analyses of manually-prepared samples of kettle curd.

Data shown in table 2 indicate also that analytical results obtained in the laboratory were more uniform than those obtained at the numerous factories,

where conditions varied more than in the laboratory and where the most desirable types of analytical equipment, such as an analytical balance and a vacuum oven, were not in all cases available. It seems probable that results of analyses and observations made under factory conditions by careful cheesemakers might not always be as uniform as those reported herein.

Changes in composition during pressing and curing. In analyses of 14 samples of unpressed curd taken from the kettle with a strainer and analyzed without having been pressed, and of the corresponding green cheese, it was found that the average percentage of fat in dry matter increased from 42.5 per cent in the curd at dipping to 46.0 per cent in the cheese. Analyses of samples of the drainage whey showed that the percentage of fat in the whey draining from the cheese gradually decreased from an average of 0.60 per cent at the beginning of pressing to about 0.20 per cent at the time when drainage ended, while the percentage of solids-not-fat in the drainage whey actually increased progressively during pressing. Analyses of the cylinder-pressed curd samples, and of whey draining therefrom, showed very similar results. Koestler (1) found that the percentage of fat in the whey draining from cheese decreased progressively from 0.59 per cent to as little as 0.10 per cent, while the percentage of total dry matter in drainage whey actually increased during pressing. The cause of the increase in percentage of fat in dry matter in cheese during pressing evidently lies in the fact that the loss of solids-not-fat from cheese during drainage is relatively great in comparison with the loss of fat; and therefore the ultimate composition is influenced by the extent of drainage.

TABLE 3

Data showing comparative average composition of curd and factory cured Swiss cheese; curd analyses made by two different methods

Method	Samples		Fat	Moisture	Fat in dry matter
	Number	Source			
1. New, samples prepared by mechanical pressing	458	Curd	% 26.07	% 42.49	% 45.33
		Cured cheese	27.63	38.84	45.18
2. Old, samples prepared manually	136	Curd	25.47	44.04	45.51
		Cured cheese	27.55	39.30	45.39

The relationship of the composition of the curd, as determined by the new as well as by the old method, with that of the cured cheese is shown in the data in table 3. The percentage of moisture was somewhat higher in the samples of curd than in the cured cheese. The average percentage of moisture found in the curd samples was lower for the new method than for the older one. The average fat-in-dry-matter values for all samples of curd were in general slightly higher than those for all samples of cured cheese.

Results of the present work confirm an observation made by Price (2), who found that when the fat-in-dry-matter value of the curd was unusually low, the fat-in-dry-matter value of the corresponding cheese tended to be slightly higher, but when the fat-in-dry-matter value of the curd was unusually high the fat-in-dry-matter value of the corresponding cheese tended to be slightly lower. We found that among those curd samples whose values were below 43 per cent the average value for the corresponding cured cheese was 0.43 per cent higher, and among the curd samples whose values were above 48 per cent the average value for the corresponding cured cheese was 0.84 per cent lower. The point at which the two trends converged was approximately 44 per cent.

In order to formulate a comparative basis for estimating composition of cured cheese by means of data on composition of kettle curd or of green cheese, analyses were made of samples taken at each of these three stages from 145 laboratory cheeses and 18 factory cheeses. Resulting data are shown in table 4. It was found that the percentage of moisture was in all cases slightly greater in the cylinder-pressed curd samples than in the green cheese; also that the percentage of fat in dry matter was usually slightly less in the curd samples than in the green cheese, but greater than in the cured cheese.

TABLE 4

Data showing average composition of Swiss cheese kettle curd and corresponding cheese, and changes in composition during curing

A. 145 laboratory cheeses each weighing about 60 pounds

	Fat	Moisture	Fat in dry matter	Salt
	%	%	%	%
Kettle curd, pressed	27.02	41.53	46.21
Cheese, 1 day old	28.64	38.44	46.52	0.03
Cheese, cured 3 months.....	29.29	36.45	46.09	0.76
Changes during curing.....	+ 0.65	- 1.99	- 0.43	+ 0.73

B. 18 factory cheeses each weighing about 180 pounds

	Fat	Moisture	Fat in dry matter	Salt
	%	%	%	%
Kettle curd, pressed	25.60	42.94	44.87
Cheese, 1 day old	27.37	39.13	44.96	0.03
Cheese, cured 2½ months	27.55	38.25	44.62	0.63
Changes in curing	+ 0.18	- 0.88	- 0.34	+ 0.60

The absolute percentage of fat in the cheese increased consistently during curing. This increase is undoubtedly caused by the fact that shrinkage in curing involves principally a loss of moisture without a proportional loss of fat or of total solids. There was a consistent decrease in percentage of

fat in dry matter during curing—a decrease that was practically accounted for by the increase in dry matter resulting from the absorption of salt. The fact that the average percentage of fat in dry matter decreased by about 0.3 to 0.45 per cent during curing indicates that in order to have some assurance of securing a given average percentage of fat in dry matter in cured cheese it is necessary to have a slightly larger percentage in the kettle curd and in the green cheese.

Calculations based on percentage of fat in kettle milk. Since the fat test of the kettle milk is the only basis of prediction in many factories, it is desirable to determine how accurately the percentage of fat in dry matter in the cheese can be predicted from a knowledge of the percentage of fat in the milk. Therefore, calculations were made by two different methods to show the extent to which the fat-in-dry-matter value of each cheese varied or deviated from the average value, for cheese made from milk of a given fat percentage.

Method 3, based on averages for all cheese sampled: Data for 729 factory cheeses, on which milk fat tests were available, were divided into groups according to the percentage of fat in the standardized kettle milk, and the average percentage of fat in dry matter in cheese in each group was determined. Resulting data are shown in line A, figure 3. The extent to which the fat-in-dry-matter value of each cheese in a group differed from the average value for that group was then calculated. Data showing the distribution of these differences are given in Method 3, table 5.

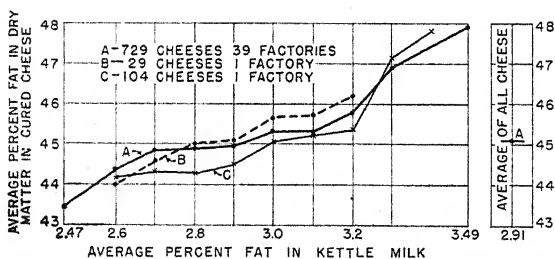


FIG. 3. Data showing average percentage of fat in dry matter in Swiss cheese made from milks of different fat percentages.

Method 4, based on averages for cheese sampled in each of 39 factories, calculated for each factory separately: Data for each factory were first divided into groups according to the percentage of fat in the standardized kettle milk used in each cheese sampled in that factory, and the average percentage of fat in dry matter in cheese in each group was determined for that factory. Resulting data for each of two representative factories are shown in lines B and C, respectively, in figure 3. The extent to which the fat-in-dry-matter value of each cheese sampled in each factory differed from the average value in that factory, for milk of a given fat percentage,

was then calculated. The differences, after being determined for each factory, were then combined and calculated to indicate their distribution, shown in Method 4, table 5.

TABLE 5

Data showing relative accuracy of calculations based on percentage of fat in kettle milk in estimating the percentage of fat in dry matter in Swiss cheese (729 factory cheeses)

Method of calculation	Percentage of predicted values falling within specified limits of variation from average values found in cured cheese				
	0 ± 0.5	0 ± 1.0	0 ± 1.5	0 ± 2.0	0 ± 2.5
3	% 26.2	% 47.3	% 62.5	% 75.8	% 83.2
4	34.6	60.5	77.8	88.5	94.6

Method 3—Based on averages for all cheese sampled; average deviation, 1.40 per cent; standard deviation, 1.76 per cent.

Method 4—Based on averages for cheese sampled in each of 39 factories, calculated for each factory separately; average deviation, 0.97 per cent; standard deviation, 1.26 per cent.

The results shown in table 5 indicate that standardization based only on the percentage of fat in the cheese milk yielded less precise results for all of the samples as a whole (Method 3) than were obtained in samples in each individual factory considered separately (Method 4). It is to be expected that the number of variables which tend to influence the composition of cheese (such as variations in solids-not-fat content of milk and in fat losses in the making process) is smaller in any one factory than in several factories located in different areas.

The results shown in table 5 indicate also that standardization based only on the percentage of fat in the milk yielded less precise results than were secured by the new, pressed-curd-analysis method (Method 1) for which data are shown in table 2.

The fact that a rather consistent relationship exists between the average percentage of fat in dry matter in the curd and that in the cured cheese (tables 3 and 4) indicates that the percentage of fat in the milk can be used to predict the percentage of fat in dry matter in the curd, and hence is a useful tool in the control of composition.

Our results show, however, that the average percentage of fat in kettle milk required to produce a given average percentage of fat in dry matter in cheese varied seasonally. Tabulations of data on 729 cheeses show that the average milk fat tests which yielded an average of 45 per cent fat in dry matter in the cheese, tabulated by months, were as follows: March, 2.81; April, 2.74; May, 2.75; June, 2.85; July, 2.81; August, 2.82; September, 3.00; October, 3.06; November, 3.08; and December, 3.00. (Data for January and February were not available.) Apparently the percentage of fat in the

standardized milk needs to be increased during the fall and winter months to maintain uniform composition in the cheese.

The results presented above indicate the approximate limits within which it should be possible to regulate, by means of analytical control, the fat in dry matter in Swiss cheese. However, if abnormal conditions (such as the occurrence of mastitis milk) are present or if the analytical facilities available are not adequate for effective control, the results of standardization may not be as uniform as those shown herein.

For efficient standardization it is essential that, in each factory, the milk in each kettle be tested and standardized; it is necessary also that tests be made frequently of the composition of kettle curd and of cured cheese, and that a set of data be prepared in the manner shown in figure 3, to be used in adjusting the percentage of fat in the milk.

Studies of the casein-fat ratio of milk are omitted from this report for the reason that tabulations of such data have not shown, for any one factory, that it is a more accurate basis than the fat test alone for predicting composition of cheese.

SUMMARY

Tabulations of analytical and commercial grading data on 844 factory Swiss cheeses show that the highest average quality was found in cheese containing from 45 to 46 per cent fat in dry matter.

Tabulations of data on 30 pairs of laboratory cheese indicate that when the body of the cheese is relatively firm the presence of a slightly higher proportion of fat tends to improve the quality.

A new method is presented for securing and preparing, in a uniform manner, pressed samples of curd from the Swiss cheese kettle, analyses of which provide a means of estimating the percentage of fat in dry matter in the cheese. By this method, fat in dry matter in the cured cheese was estimated within one per cent in slightly more than two-thirds of the cases.

For efficient standardization it is suggested that, in each factory, the milk in each kettle be tested and standardized; that, for control purposes, pressed samples of kettle curd be secured frequently and analyzed for percentage of fat in dry matter; and that similar analyses be made frequently on samples of cured cheese.

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AN ANALYSIS OF CONTESTANT JUDGMENTS IN THE SCORING OF DAIRY PRODUCTS WITH A STUDY OF SOME FACTORS WHICH MAY AFFECT THEM

G. M. TROUT, CH., WILLIAM WHITE, P. A. DOWNS, M. J. MACK
AND E. L. FOUTS

Committee on Judging Dairy Products, A.D.S.A.

Questions frequently arise in the judging of dairy products relative to the effect of some factors on the efficiency of judging, particularly when a specific number of samples are judged by several groups over an extended period. Inasmuch as 2520 contestant-sample and 8190 contestant-item judgments were involved in the 1940 Students' National Contest in the Judging of Dairy Products, these data seemed to furnish an opportunity for studying what effect such factors as fatigue, order of judging, and quality of product had upon the reliability of judgments.

Sixty-three men comprising 21 teams from state colleges and universities¹ judged 10 samples each of butter, cheese, milk and ice cream. The sample judgments totaled 630 for each product, giving a sum of 2520 for the contest. In arriving at the sample judgment each contestant passed judgment on 40 items for butter (Package allowed perfect score) ; 30 items for cheese (Finish allowed perfect score) ; 30 items for milk ; and 30 items for ice cream, giving a total item judgment of 2520 for butter, and 1890 each for cheese, milk and ice cream.

In dairy products judging the contestant's grade is a negative grade, being in part the difference between the official score and the contestant's score, and in part, the grade, not exceeding one point per score card item, based upon the contestant's ability to describe the quality as indicated and described by the official judge. Obviously, the contestant with the lower grade has the higher rank in judging ability, inasmuch as his judgment is closer to the official judgment.

Grouping of Contestants. The 63 contestants were divided into four groups so that no two team members were in any one group. Although a definite sequence of numbers was followed in grouping the contestants, the relegation of contestants to groups actually represented random sampling, inasmuch as team members and teams lined up of their own volition for contestant number assignment. In this discussion, the groups will be designated as A, B, C, and D. Groups A, B, and C each had 16 members, whereas group D had 15 members. Numbers 1, 5, 9, etc. were in group A ; 2, 6, 10, etc. in Group B ; 3, 7, 11, etc. in Group C ; and 4, 8, 12, etc. were in Group D.

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¹ Connecticut, Cornell, Illinois, Iowa, Kansas, Maryland, Massachusetts, Michigan, Minnesota, Mississippi, Nebraska, New Hampshire, New Jersey, Ohio, Pennsylvania, Purdue, Tennessee, Texas Technological, Vermont, Virginia Polytechnic, and Wisconsin.

Order of Scoring. The order of scoring each product was carried out according to the schedule in table 1.

TABLE 1

The order of scoring and period in which scoring was done by each group

Group	Product assigned to group during			
	1st period	2nd period	3rd period	4th period
A	Cheese	Butter	Ice cream	Milk
B	Butter	Ice cream	Milk	Cheese
C	Ice cream	Milk	Cheese	Butter
D	Milk	Cheese	Butter	Ice cream

Thus, three groups, A, B, and D scored ice cream following butter; three groups, A, B, and C, scored milk after ice cream; three groups, B, C, and D scored cheese after milk; and three groups, A, C, and D scored butter after cheese. The order of products scored by each group was butter, ice cream, milk, cheese, and so on regardless of the product scored first.

Comparative Abilities of the Groups. On the basis of total grades for all products, some differences were noted in the scoring abilities of the various groups, or some factors were operating that influenced the judging. The grades are shown in table 2.

TABLE 2

The scoring abilities of the various groups as shown by the total grades

Group	Total grade per group in the scoring of				
	Butter	Ice cream	Milk	Cheese	All products
A	376.75 ^{2*}	763.45 ³	529.95 ⁴	580.40 ¹	2250.55
B	363.75 ¹	790.50 ²	504.25 ³	553.50 ⁴	2211.50
C	373.50 ⁴	704.00 ¹	533.20 ²	464.50 ³	2075.20
D†	392.80 ³	769.28 ⁴	533.71 ¹	548.00 ²	2243.79

* Numbers 1, 2, 3, 4, indicate the period during which the scoring was done.

† Grades in Group D, composed of 15 contestants, were weighted to compare with those of Groups A, B, and C, composed of 16 members each.

On the basis of the above scores each group would have ranked in the scoring of the several products as shown in table 3.

TABLE 3

The standings of the various groups in the judging of butter, ice cream, milk, and cheese

Group	Standing in the judging of				Total	Rank all products
	Butter	Ice cream	Milk	Cheese		
A	3rd	2nd	2nd	4th	11	3rd
B	1st	4th	1st	3rd	9	2nd
C	2nd	1st	3rd	1st	7	1st
D	4th	3rd	4th	2nd	13	4th

From these scores and rankings it would appear that Groups B and C were slightly superior to the other two groups in judging ability.

Distribution of First 10 Ranking Contestants per Group. The sum of the distributions of the first ten winning individuals in each product according to group presented in table 4 shows that 6 and 15 respectively were in Groups D and C, or 21 out of the possible 40. Thus it would appear that the groups were about equally divided as to abilities except possibly Group C in which were 6 of the 10 ranking individuals in scoring ice cream. Possibly this higher number in one group in the scoring of ice cream may have been due to the period in which the scoring was done.

TABLE 4

Distribution of the ranking 10 individuals in the scoring of each product as to group

Group	Number of first ten men in the scoring of products of each group				Total
	Butter	Ice cream	Milk	Cheese	
A	3	1	4	3	11
B	3	2	2	1	8
C	2	6	3	4	15
D	2	1	1	2	6

The distribution of the same individuals as to the period in which the judging was done is shown in table 5.

TABLE 5

Distribution of the ranking 10 individuals in the scoring of each product according to period in which they judged

Period	Number of first 10 men in the scoring of products as to period in which judging was done				Total
	Butter	Ice cream	Milk	Cheese	
1st	3	6	1	3	13
2nd	3	2	3	2	10
3rd	2	1	2	4	9
4th	2	1	4	1	8

From the above totals, based only on the ranking 10 individuals, it would appear that the best judging was done during the first period, after which the judging was less effective. However, if the number of ranking individuals in the scoring of ice cream per period is disregarded, then the numbers of ranking individuals per period are 7, 8, 8, and 7, respectively. Apparently, from the number of the first 10 ranking individuals in the scoring of ice cream, the first period was conducive to the best judging. In summation, from the data at hand it appears that the groups were fairly well balanced as to judging ability.

The Effect of the Preceding Product on the Efficiency of Scoring. Con-

testants frequently give expression to the thought they would prefer to score one particular product before scoring another, probably on the assumption that it is more difficult to score some one product after having scored another, for instance, scoring milk after previously having scored cheese. The grades, therefore, were compiled in table 6 according to period to note what effect the previous product had on the judging. A study of the data shows that Groups A, D, and C each had higher grades in scoring butter than Group B. Group B started the contest by scoring butter first, whereas, each of the other three groups scored butter in turn after previously having scored cheese. The grades of groups A and C are only slightly higher and are likely not of any significance, but may indicate a trend. It must be borne in mind, however, that during the period of the contest, lasting approximately 5 hours, the butter was exposed to room temperature, so that the condition of the butter, particularly as to body, may not have been the same for each group. This factor may have offset any advantage or disadvantages of the previous product. However, the comparatively high grade of group D scoring butter in the third period would seem unexplainable. It is not unlikely that the lower grade of the group scoring butter first may have been due in part to psychological factors.

In the scoring of ice cream three groups, A, B, and D scored ice cream following butter. Here again the group scoring the product first had the lowest grade, with a rather wide margin below Groups A and B. Inasmuch as the ice cream was held in an electric refrigerator during the contest, the condition of the product, except possibly the sample exposed to show melting quality, would not have changed as did butter.

On the other hand, three groups, A, B, and C, scored milk after having scored ice cream and had equal to or slightly lower grades than Group D, which scored milk first. Since fresh samples of tempered milk were set out for each group, these differences cannot be explained on changes in the sample during the period of the contest. If not the sequence of the scoring, some other factors must have had an influence.

Finally, three groups, B, C, and D, scored cheese after having scored milk previously. From the standpoint of having the organs of the mouth in optimum condition for tasting, and by reason of contrast in intensity of flavor between products, such an arrangement would seem to be ideal for scoring cheese. And apparently it proved to be, for the three groups, instead of having higher grades than the group scoring the product first actually had materially lower grades. The cheese samples, like the butter, were exposed to room temperature during the entire contest which may have been a factor resulting in the lower grades.

The Effect of the Period of Judging on the Efficiency of the Contestant. The total grades per period presented in table 6 indicated that the scoring per period as the contest progressed was fairly constant. However, some

TABLE 6

The total grade per product according to the period of judging

Product	Total grades according to the period of judging				
	First	Second	Third	Fourth	Total†
Butter	363.25 B*	376.75 A	368.25 D (392.80)	373.50 C	1481.75
Ice cream	704.00 C	790.50 B	763.45 A	721.20 D (769.28)	2979.15
Milk	500.35 D‡ (533.71)	533.20 C	504.25 B	529.95 A	2067.75
Cheese	580.40 A	513.75 D (548.00)	464.50 C	553.50 B	2112.15
All products	2148.00	2214.20	2100.45	2178.15	8640.80

* Group.

† Actual total, not weighted.

‡ Numbers in parentheses weighted.

differences were noted. From the sum of grades per period, it is evident that the best judging was done in the third period followed closely by that in the first period, the poorest judging being done in the second and fourth periods, respectively. The poorest judgment in the second period, might be due to psychological factors such as a general "let down" of nervous energy or to lack of concentration resulting from retention of thought of poor judgment in the first period. The poorer judgment in the last period possibly might be explained by fatigue, or by less keenness in checking details, re-scoring and general desire to finish. Hardly may the poorer judgments of the second and fourth periods be explained on the change in the products for that factor would have held as well in the third period when the best judging was done.

A Comparison of Average Grades on a Single Score Card Item According to Product and According to the Period of Judging. The average contestant grade per score card item according to group and period of judging is shown in table 7. With the exception of sediment in milk only those items common to several products were included. The average contestant grade will be seen to have varied according to item, product, and period in which the judging was done, thus showing very few trends throughout the contest. However, several data are worthy of special consideration. As a general observation, the grades on flavor criticisms are of about the same value regardless of product, group, or time of judging, with slightly lower values for milk, thus showing that the contestants know the flavor criticism of each product about equally well and probably those of milk best of all. However, the range in grades on flavor score between products would indicate that contestants were at more of a loss in evaluating than in designating the flavor, particularly in scoring flavor of ice cream and milk which apparently caused

more trouble than butter and cheese. It appears that the contestants as a whole had too high an average grade on flavor score of milk considering the low grades on flavor criticism. In other words, it would seem necessary to standardize the score of milk per specific flavor criticism within limits so that the contestant would not be at so great a loss in placing a value upon the flavor encountered.

TABLE 7

The average contestant grade per score card item by groups according to the period of judging

Product	Items	Average contestant grade of a group per sample when judging was done in the following period			
		First	Second	Third	Fourth
Butter	Flavor score	1.14 B	1.18 A	1.23 D	1.03 C
Ice Cream	" "	1.99 C	2.46 B	2.33 A	2.33 D
Milk	" "	2.04 D	2.12 C	1.99 B	1.95 A
Cheese	" "	1.43 A	1.20 D	0.93 C	1.18 B
Butter	Flavor criticism	0.58 B	0.65 A	0.61 D	0.61 C
Ice cream	" "	0.64 C	0.64 B	0.66 A	0.72 D
Milk	" "	0.51 D	0.56 C	0.56 B	0.61 A
Cheese	" "	0.68 A	0.64 D	0.61 C	0.69 B
Butter	Body and texture score	0.19 B	0.20 A	0.22 D	0.30 C
Ice cream	" " " "	0.92 C	1.05 B	0.95 A	0.96 D
Cheese	" " " "	0.90 A	0.90 D	0.72 C	0.89 B
Butter	Body and texture criticism	0.31 B	0.30 A	0.34 D	0.36 C
Ice cream	" " " "	0.58 C	0.58 B	0.57 A	0.61 D
Cheese	" " " "	0.52 A	0.53 D	0.48 C	0.52 B
Milk	Sediment	0.57 D	0.52 C	0.43 B	0.48 A
	Total	13.00	13.53	12.63	13.24

In scoring the body and texture of butter, ice cream, and cheese, the average contestant grade was higher for body and texture score of ice cream and cheese than for butter, since 9, 10 and 2 samples, respectively, were criticized in this respect, and since closer scoring is usually done on body and texture of butter than on ice cream and on cheese. The slight increase of average contestant grade per period in the *score* for body of butter might possibly be explained in changes occurring in the scoring condition of the butter during the contest. Likewise, there was a slight increase in the average contestant grade on body and texture *criticism* from the first to the fourth period of judging, whereas the average grades on criticisms for body and texture of ice cream and cheese remained fairly constant, from one period to another. However, it is extremely doubtful if any significance may be attributed to these increases due to changes in the product. For example, the average contestant grade for sediment in milk, a fixed factor, ranged from 0.57 in the first period to 0.43 in the second period. This difference must be explained from the standpoint of the student, not in changes in the item scored.

Comparison of Average Contestant Grades on a Single Item per Sample per Period. A study of the average contestant grade per sample presented in table 8 and 9 shows considerable variation in the contestant grades on flavor score between samples, particularly on flavor scores of milk and ice cream. The average contestant grade on two samples of milk, numbers 4, which was rancid and feedy, and 8, which was oxidized, were 4.22 and 3.48, respectively; that on the two garlic samples numbers 2 and 6 were 2.89 and 2.75, respectively. Likewise, in the scoring of flavor of ice cream high average grades were encountered with some samples. Apparently, the contestants for the most part were conservative and scored within a narrower range than did the official judges. If the range of official scores is considered, then the highest average grade per point of range was made in the scoring of flavor of cheese with an average of 0.40.

In the scoring of body and textures of butter, cheese and ice cream, comparatively high grades were secured with samples 3 and 9 of butter; samples 8, 9, and 10 of cheese; and samples 5, 6, and particularly 9 of ice cream thus indicating conservatism on the part of the student. It is not beyond possibility, however, that the official judges may have been somewhat severe in scoring these samples.

SUMMARY AND CONCLUSIONS

A critical study was made of 2520 sample judgments in the scoring of butter, cheese, milk and ice cream in the 1940 Students' National Contest in the Judging of Dairy Products, involving 2520 item judgments for butter and 1890 each for cheese, milk and ice cream, a total of 8190. The 63 contestants, assigned to four groups, judged the four products during four periods of 55 minutes each.

The sequence of product judging, namely, butter, ice cream, milk, and cheese was followed throughout. The effect of the previous product on scoring was, therefore, constant except for the starting period.

The two groups scoring butter and ice cream in the first period had slightly lower grades than the groups which scored those products in the second, third, or fourth periods. On the other hand the three groups which scored cheese after having scored milk had a much lower grade than the group which scored cheese first. The best judging of milk was done in the third period.

Excitement and fatigue appeared to be factors of little consequence as indicated by the total scores of the various periods. Slightly lower grades, showing superior judging, were obtained in the third and first periods, respectively; the highest grades, showing poorest judging, being made during the second period.

Higher average grades were made on scoring flavor of milk and ice cream than on scoring flavor of butter and cheese; whereas, little difference was noted between the average grades on criticisms for the four products.

TABLE 8

*The average contestant grade on flavor score and on flavor criticism
per sample per product*

Sample No.	Official flavor		Average contestant grade per sample on	
	Criticism	Score	Score	Criticism
Butter				
1	Coarse	37.0	1.49	0.80
2	Old cream, coarse	35.0	1.00	0.67
3	Old cream, cheesy, neut.	32.0	1.66	0.51
4	38.0	1.07	0.68
5	Coarse, old cream	36.5	0.77	0.64
6	Unclean, old cream, neu- tralized	35.5	0.92	0.46
7	38.0	1.13	0.78
8	Coarse	37.0	0.70	0.56
9	Old cream	34.0	0.99	0.59
10	Old cream, neutralized	33.0	1.75	0.46
		Range 6.0	Avg. 1.15	0.62
		Avg. grade per unit range 0.19		
Cheese				
1	Flat	39.0	0.90	0.67
2	Unclean, acidy	38.5	1.15	0.61
3	Unclean, acidy	38.5	1.02	0.63
4	Unclean, acidy	38.5	0.91	0.67
5	Unclean, acidy	38.0	0.93	0.63
6	Flat	39.0	1.40	0.94
7	Acidic	37.0	1.31	0.52
8	Unclean	36.0	1.76	0.58
9	Unclean	37.0	1.40	0.69
10	Unclean	37.0	1.28	0.65
		Range 3.0	Avg. 1.21	0.66
		Avg. grade per unit range 0.40		
Milk				
1	Sl. cooked	22.0	0.62	0.44
2	Garlic	14.0	2.89	0.53
3	Unclean	19.0	1.90	0.93
4	Rancid, feed	16.0	4.22	0.75
5	Feed	21.0	1.22	0.46
6	Garlic	16.0	2.75	0.70
7	23.0	0.74	0.33
8	Oxidized	15.0	3.48	0.22
9	Sl. cooked	22.0	1.58	0.67
10	Sl. cooked	21.5	0.92	0.60
		Range 9.0	Avg. 2.03	0.56
		Avg. grade per unit range 0.22		
Ice Cream				
1	45.0	1.50	0.78
2	Lacks fine flavor	44.0	1.21	0.56
3	45.0	2.37	0.93
4	Unnatural, metallic, old ingredient	38.0	3.02	0.55
5	Lacks fine flavor	42.5	2.03	0.76
6	Old ingredient, oxidized	39.0	3.05	0.65
7	Unnatural, old ingredient	38.5	3.04	0.56
8	Lacks fine flavor, lacks sugar	43.5	1.00	0.59
9	Old ingredient, storage feed, oxidized	36.0	3.69	0.47
10	Lacks fine flavor	44.5	1.92	0.71
		Range 9.0	Avg. 2.22	0.65

TABLE 9

The average contestant grade on body and texture score and on body and texture criticism per sample per product

Sample No.	Official body and texture		Average contestant grade per sample on	
		Score	Score	Criticism
Butter				
1	25.0	0.09	0.08
2	25.0	0.22	0.24
3	Crumbly	24.5	0.43	0.81
4	25.0	0.07	0.13
5	25.0	0.34	0.48
6	25.0	0.14	0.16
7	25.0	0.14	0.22
8	25.0	0.08	0.16
9	Crumbly	24.5	0.50	0.75
10	25.0	0.30	0.29
		Range 0.5	Avg. 0.23	0.33
		Avg. grade per unit range 0.46		
Cheese				
1	Open	29.0	0.58	0.32
2	Mealy, open	28.5	0.42	0.58
3	Mealy, open	28.0	0.71	0.55
4	Pasty, open	28.0	0.54	0.48
5	Mealy, open	28.0	0.58	0.50
6	Open, sw. c. holes	28.5	0.64	0.46
7	Mealy	28.0	0.78	0.74
8	Gassy, yeast slits, curdy	26.0	1.50	0.65
9	Gassy, yeast slits, curdy	26.0	1.48	0.50
10	Gassy, yeast slits, curdy	26.0	1.30	0.40
		Range 3.0	Avg. 0.85	0.52
		Avg. grade per unit range 0.28		
Ice Cream				
1	24.5
	or curdy	24.0	0.60	0.53
2	24.5	0.71	0.75
3	Curdy	24.0	0.58	0.55
4	24.5
	or curdy	24.0	0.71	0.70
5	Icy, weak	22.0	1.27	0.55
6	Icy, gummy (soggy)	22.0	1.15	0.52
7	Icy	22.0	0.98	0.30
8	24.5	0.73	0.84
9	Buttery, gummy (soggy), icy, curdy, does not melt	20.0	2.50	0.60
10	Wheys off, curdy, crumbly, does not melt	23.5	0.52	0.57
		Range 4.5	Avg. 0.97	0.59
		Avg. grade per unit range 0.21		

Contestants appeared to be more conservative in scoring strong off-flavors of milk and ice cream than the official judges.

The change in body and texture of the products during the progress of

the contest as affecting its scorability would seem to be of minor importance, dependent upon the product; changes in cheese being of little consequence; changes in ice cream negligible, except possibly for melting quality; whereas, changes in body of butter were such as to increase slightly the average contestant grades per period of judging.

The distribution of the 10 ranking individuals per period was quite uniform except in the judging of ice cream when 6 of the 10 ranking individuals scored ice cream in the first period.

IN VIVO STUDIES OF HYDROGEN ION CONCENTRATIONS IN THE RUMEN OF THE DAIRY COW

VEARL R. SMITH

Department of Dairy Husbandry, Oregon State College

INTRODUCTION

The rumen of herbivorous animals plays an essential and important role in the digestive process although no digestive juices are secreted in the rumen. Moisture, body heat, and rumen motility provide an excellent environment for the fermentation and maceration of the coarse, bulky, and fibrous material that constitutes the major portion of the herbivore's diet. Cellulose, the main constituent of the crude fiber of plant foods, is utilized by herbivorous animals. Dukes (2) states that bacteria are the chief agencies involved in cellulose digestion and suggests that bacteria ferment cellulose to glucose. For optimal bacterial activity, the hydrogen ion concentration needs to be within a certain range. With possible acid production from carbohydrate fermentation, the pH of rumen ingesta should be influenced by types of feed consumed, which in turn may have a regulatory action on bacterial activity.

Kick and co-workers (3) reported studies with steers on the pH of rumen ingesta. Their determinations varied with rations fed from pH 5.5 to 7.7 with the most alkaline reaction on alfalfa hay alone.

Monroe and Perkins (4) made *in vitro* determinations of the hydrogen ion concentration of rumen ingesta. The pH determinations were made with a Leeds and Northrup portable potentiometer with a saturated calomel half cell and a quinhydrone electrode. Rumen contents were sampled in six different localities and pH readings were taken on uncentrifuged liquid expressed from the ingesta. They gave an average pH value between 6.83 and 7.01 when their animals were fed roughages such as corn and A.I.V silage, and alfalfa hay. A slightly more acid reaction was obtained when the animals were pastured on bluegrass and alfalfa. They reported rumen contents of slaughtered animals to have an average pH of 7.34.

Previous work done at this laboratory showed that some body fluids undergo rapid changes in pH when exposed to air. For this reason it was thought that *in vivo* determinations would be nearer the actual hydrogen ion concentration.

EXPERIMENTAL ANIMALS AND METHODS

The experimental animals used in this study were two Holstein cows with

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permanent rumen fistulas. Both animals were kept in stanchions. Water was available at all times from fountains. One of the animals was used as a nurse cow and the other was milked during the experimental period. The fistulas were not kept closed and a small amount of ingesta was lost when the animals were lying down.¹

A Beckman pH meter with a glass electrode assembly constructed for such purposes was used in the work. The customary potassium chloride calomel electrode in this assembly is replaced by a silver-silver chloride electrode.

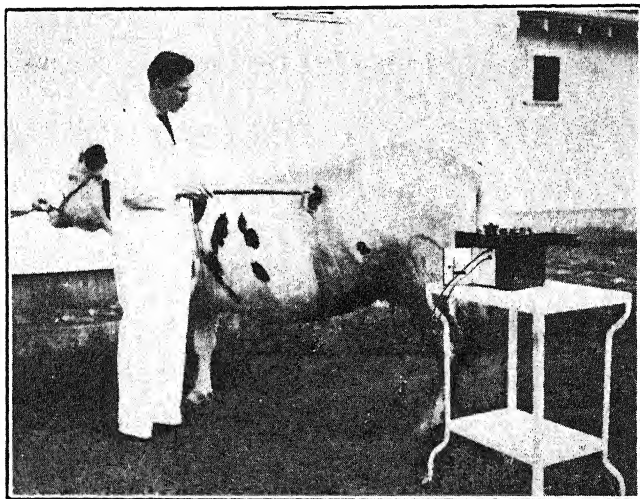


FIG. 1. Illustrates method of making pH readings and equipment used.

This electrode is located on the body of the glass electrode some four inches from the ground portion and connects through the glass to the lead wire provided with a phone tip. The electrode and extension jacket measure 23.5 inches, and are connected to the pH meter by a ten-foot lead.

The electrode was inserted into the rumen, and PH readings were made in six different localities. Readings designated as front were taken in the immediate vicinity of the cardia approximately two inches below the ingesta level. Deep front readings were made with the electrode near the reticulum and a few inches above the floor of the rumen. The middle readings were made in the dorsal sac near the surface and the deep middle readings from the ventral sac an inch or two above the floor of the rumen. Rear and deep

¹ In this study the rumen fistulas were not kept closed because it was found more convenient to take readings in various locations in the rumen. Since submitting this paper for publication, additional studies have been made which show that the pH of the rumen was slightly more acid (0.30) with a closed fistula.

rear readings were made in the dorsal and ventral posterior blind sacs of the rumen respectively.

The experimental period on alfalfa for animal No. 1 was two weeks previous to the corresponding period for animal No. 2. Alfalfa hay and beet pulp experimental periods were simultaneous for the two animals. In order to insure a homogenous ingesta, beet pulp was fed one week prior to the beginning of the experimental period. Readings were made three times a day over a period of five successive days for each animal on each ration. In addition, there were 24-hour periods at the end of the five-day experimental periods in which readings were made every two hours.

RESULTS OBTAINED

Results of pH readings when the animals were on a sole alfalfa hay ration are given in table 1. Means for the various periods represent 30 readings and an overall mean 90 readings. The mean for the 24-hour period is for 72 readings. The readings on animal No. 1 ranged from pH 5.65 to 6.78. The mean pH readings of the 7:30 A.M., 12:30 P.M., and 5:30 P.M. were pH 6.44, 6.22, and 6.13, respectively. The overall mean or mean of all readings for animal No. 1 was pH 6.26. The 24-hour period had a mean pH of 6.29. The range in pH for animal No. 2, when receiving alfalfa hay alone, was 5.85 to 6.85. Means for the various hours are as follows: 7:30 A.M., pH 6.43; 12:30 P.M., pH 6.24; and 5:30 P.M., pH 6.20, with an overall mean of pH 6.29. Mean of the 24-hour period is pH 6.31.

TABLE 1

Comparison of pH means when animals were fed alfalfa hay alone

Experimental animal	pH means of the various periods				
	7:30 A.M.	12:30 P.M.	5:30 P.M.	Overall	24-hour period
No. 1	6.44	6.22	6.13	6.26	6.29
No. 2	6.43	6.24	6.20	6.29	6.31

Table 2 gives the summary of pH readings when the animals received 20 pounds of molasses beet pulp in addition to alfalfa hay ad libitum. For animal No. 1 there was a range in readings from pH 5.29 to 6.54. The mean pH of 7:30 A.M. readings is 6.15; of 12:30 P.M., pH 5.82; and of 5:30 P.M., pH 5.93, with an overall mean of pH 5.97. The 24-hour period mean is pH 6.07.

The range of readings for animal No. 2 was pH 5.55 to 6.59, a mean pH of 6.14 for the 7:30 A.M., 6.01 for the 12:30 P.M., 5.95 for the 5:30 P.M. readings, and an overall mean of pH 6.03. On the readings over the 24-hour period there was a mean pH of 6.07.

From these data it can be seen that the results are consistent. The pH was highest at the 7:30 A.M. readings and lowest at 5:30 P.M. The feeding

TABLE 2

Comparison of pH means when animals were fed alfalfa hay and beet pulp

Experimental animal	pH means of the various periods				
	7:30 A.M.	12:30 P.M.	5:30 P.M.	Overall	24-hour period
No. 1	6.14	6.01	5.95	6.03	6.07
No. 2	6.15	5.82	5.93	5.97	6.07

of beet pulp increased the acidity of the ingesta appreciably. This is evidenced when the pH readings on the beet pulp plus alfalfa hay are compared to the readings of hay alone.

Table 3 presents the comparison of *in vitro* and *in vivo* determinations.

TABLE 3

Comparisons of in vitro and in vivo pH determinations of rumen ingesta

Determination made	Locations from which samples or readings were taken					
	Front	Deep front	Middle	Deep middle	Rear	Deep rear
Experimental Animal No. 1						
<i>In vitro</i>	6.90	6.55	6.57	6.43	6.50	6.57
<i>In vivo</i>	6.32	6.32	6.32	6.28	6.28	6.25
Experimental Animal No. 2						
<i>In vitro</i>	6.88	6.49	6.61	6.23	6.17	6.10
<i>In vivo</i>	6.35	6.40	6.10	6.02	5.83	6.16

The *in vitro* pH determinations were made on liquid from ingesta taken from the same parts of the rumen where the *in vivo* readings were made. Samples of ingesta were taken immediately after *in vivo* readings. The *in vitro* determinations were completed within 30 minutes after the samples were taken. The customary potassium chloride calomel electrode was used in making the *in vitro* readings. Table 3 shows that the *in vitro* values were more alkaline than the *in vivo* values in all cases except one.

TABLE 4

Mean pH readings of rumen ingesta in various parts of the rumen

Front	Deep front	Middle	Deep middle	Rear	Deep rear
6.27	6.20	6.05	6.13	6.00	6.13

Mean pH values of rumen ingesta in various parts of the rumen are given in table 4. Means are of readings on both rations and each figure represents 30 pH determinations. The front reading, which was taken near the cardia, has the highest pH value. This is logical, since the 7:30 A.M. and 5:30 P.M. readings were made shortly after the animals were fed. Dukes (2), Kick and associates (3), and Monroe and Perkins (4), assess saliva a pH of 8.00

and over. The contractions of the reticulum keep the ingesta in the cardia region well bathed with liquid ingesta. Rear and middle readings are more acid and were made in the moist ingesta; whereas the deep front, deep middle, and deep rear readings were taken in the more liquid ingesta composed of suspended material, ingested water, and salivary secretions, and were more alkaline.

Figure 2 presents graphically the means of the pH readings at two-hour intervals on animal No. 1. The animal was fed at 7:30 A.M. and 5:30 P.M. The curve representing the alfalfa hay pH readings shows a gradual increase in alkalinity up to 9:00 A.M. after which there is a downward trend until 11:00 A.M. The pH takes an upward trend from then until 5:00 P.M. With minor fluctuations, there is a gradual rise to 1:00 A.M., the high point of the period with pH 6.47. The broken line, which indicates the trend of the mean pH readings on beet pulp, has the highest pH of the day at 7:00 A.M., or thirty minutes before being fed. There is an increase in pH from 1:00 P.M. to 5:00 P.M., a decline to the low point of the day at 9:00 P.M., and a general rise to 3:00 A.M.

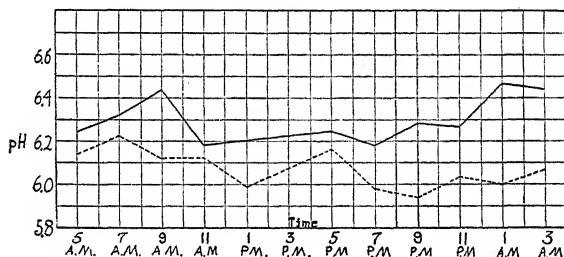


FIG. 2. Means of pH readings taken at two-hour intervals, experimental animal No. 1. Continuous line-alfalfa hay alone, broken line-alfalfa hay and beet pulp.

Figure 3 represents means for animal No. 2. The high point in the continuous line curve is at 9:00 A.M., after which there is a constant decline to 5:00 P.M., an incline to 7:00 P.M., a drop to 9:00 A.M., a slight rise at 11:00 P.M., a lowering at 1:00 A.M., and a rise at 3:00 A.M. The curve for the beet pulp was comparable to the alfalfa curve in that it reached its peak at 9:00 A.M. Another high point occurred at 3:00 P.M., followed by a general trend downward to 11:00 P.M. The curve then takes an upward swing to 3:00 A.M. A study of the graphs shows that the mean pH values on the alfalfa hay and beet pulp ration are without exception lower than the values on the alfalfa alone.

The curves do not follow any consistent pattern with respect to time of day. The fluctuations of these curves are probably influenced by large amounts of saliva entering the rumen during rumination. Schalk and Amadon (5) state that the ox secretes approximately 15 gallons of saliva per day and that 50 per cent of rumen ingesta consists of ingested water and

salivary secretions. They explain that the remasticated bolus is deposited in the anterior dorsal sac of the rumen. Saliva accompanying the bolus would have the opportunity of being mixed with rumen ingesta. Water follows the same path as the bolus and may be a factor in causing pH fluctuations.

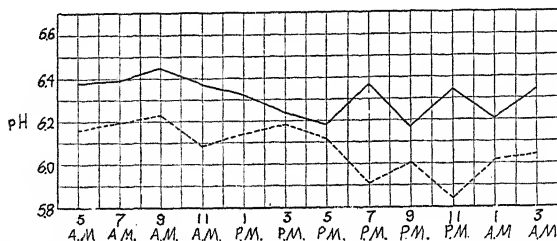


FIG. 3. Means of pH readings taken at two-hour intervals, experimental animal No. 2.

DISCUSSION

Significance of pH in the rumen is as yet unknown. Hydrogen ion concentration may be a factor in bloat in that it may provide optimum conditions for fermentation activities of bacteria and the formation of gases. Dougherty (1) has shown that gas is absorbed from the rumen and that the rate of absorption is increased with an increase of intraruminal pressure. Carbon monoxide was found to be a gas common to the rumen and was capable of producing symptoms of distress in low concentrations when ruminal pressure was increased. Later unpublished work by the same author indicates the hydrogen ion concentrations of the rumen may have a marked influence on the production and the absorption of hydrogen sulfide into the blood stream. The later-mentioned gas was thought to be an important cause of some of the symptoms of acute bloat. Raising or lowering of pH in rumen ingesta may result from the accumulation of products of bacterial activity.

Mean pH values determined in this work are lower than those reported by other workers. These lower values may be due to the fact that pH determinations were made *in vivo*. The more alkaline values for the *in vitro* determinations are possibly due to the loss of CO₂ from the *in vitro* samples.

SUMMARY

Hydrogen ion determinations were made on rumen ingesta, using as experimental subjects two cows with permanent rumen fistulas.

Readings of pH were taken three times a day over a period of five days and at two-hour intervals over a period of 24 hours for two different rations.

A mean hydrogen ion concentration of 6.27 for alfalfa hay alone and 6.00

for alfalfa hay and beet pulp was obtained over a period of five days with three-times-a-day readings.

Over the 24-hour period with two-hour-interval readings, the mean pH for the alfalfa ration is 6.30 and for the alfalfa and beet pulp ration 6.07.

Rumen ingesta fluctuate in hydrogen ion concentration throughout the day but the ingesta are generally more alkaline shortly before and after feeding.

Mean hydrogen ion concentration readings varied from pH 6.27 in the front to pH 6.00 in the rear of the rumen.

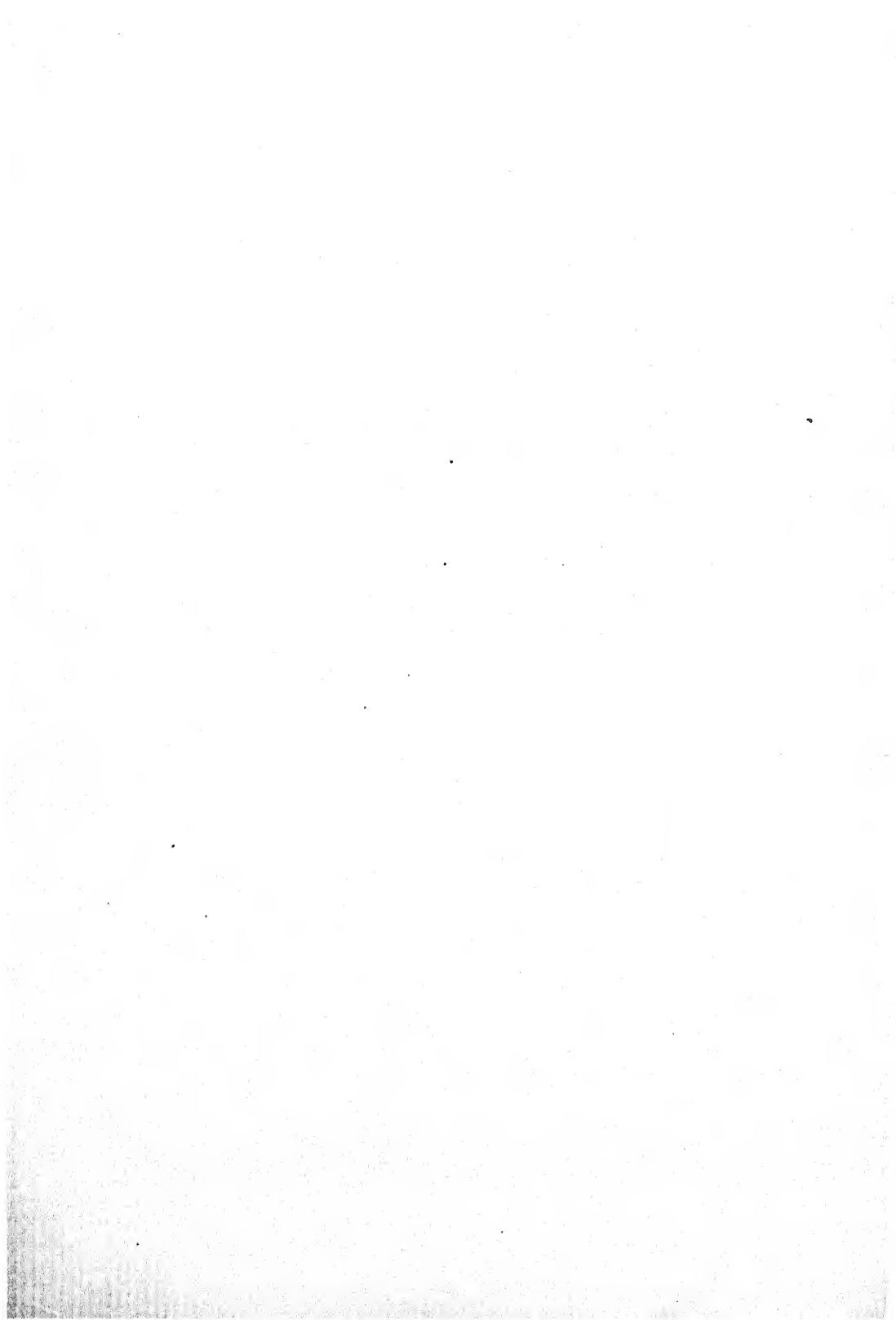
Lower pH values were obtained by *in vivo* pH determinations than by *in vitro* determinations.

ACKNOWLEDGMENTS

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THE SIGNIFICANCE OF LIPOLYSIS IN THE CURD TENSION AND RENNET COAGULATION OF MILK. I. THE ROLE OF FAT GLOBULE ADSORPTION "MEMBRANE." II. THE EFFECT OF THE ADDITION OF CERTAIN FAT ACIDS TO MILK

N. P. TARASSUK AND G. A. RICHARDSON
Division of Dairy Industry, University of California

The relatively low curd tension of fresh sweet cream buttermilk (1, 2) and the failure to clot with rennet of buttermilk obtained from certain creams having other than a natural fat globule adsorption "membrane" (3) have been attributed by Tarassuk and Palmer (3), Palmer and Tarassuk (4) to two possible factors: 1. The curd tension reducing effect of adsorption "membrane" protein as the result of its possible partial denaturation in the process of churning of cream; 2. The interference of certain fat acids with a normal clotting of milk by rennet. The fat acids are liberated by partial hydrolysis of milk fat, which, presumably takes place under certain conditions on the replacement of a natural adsorption "membrane" around the fat globules.

In the present report experimental evidence is submitted to show (a) the extent and significance of lipolysis in curd tension reduction of milk and buttermilk by fat globule "membrane" replacement and (b) the conditions under which certain free fat acids when present in milk will completely inhibit the clotting of milk by rennet.

EXPERIMENTAL

The creams having other than a natural fat globule "membrane" were prepared as described in detail previously (3). Essentially, the procedure was as follows: Fresh, pure butter fat is emulsified in an aqueous solution of the desired emulsifying agent to give the synthetic cream. By diluting this cream with normal fresh skim milk "remade" whole milk is obtained. Centrifugal separation of the "remade" cream on churning produces "remade" buttermilk.

The curd tension, surface tension, pH, . . . values, were determined as described in a previous paper (3). The official method (1936) was employed for the determination of free fatty acids and the values obtained are expressed as acid degree of fat (ml. of 1N NaOH required to neutralize the free acids in 100 grams of fat).

I. THE EFFECT OF LIPOLYSIS ON CURD TENSION AND RENNET COAGULATION OF "REMADE" MILKS

In the previous work (2, 3) dealing with the effect of fat globule "mem-

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brane" material on the curd tension of "remade" milks, the source of milk plasma for such milk was a normal raw skim milk. The employment of raw skim milk inadvertently introduced the possibility of lipolysis as suggested by the abnormally low surface tension and pH value of the "remade" buttermilks. In a later report of Palmer and Tarassuk (4) it was shown that when diglycol laurate was added to milk, or used as an emulsifying agent, the hydrolysis of the ester always took place when a raw milk was employed. The hydrolysis of diglycol laurate in milk led, under certain conditions, either to reduced curd tension or to a total inhibition of clotting by rennet. In order to evaluate 1, the effect of adsorption "membrane" material and 2, the effect of possible lipolysis on the curd tension of "remade" milks, we have repeated independently the essential experiments of Tarassuk and Palmer (3) with the additional modification of using pasteurized skim milk as the source of milk plasma for "remade" milks.

Table 1 presents the typical results obtained. In experiment I, the only difference between (a) and (b) parts was that raw skim milk was employed in (a) and identical skim milk pasteurized by holding method was used in (b). The "remade" milks in this experiment were made from the same gelatin cream.

Two important things are evident from the examination of the pH and the surface tension values of "remade" milks in table 1: 1. Whenever a pasteurized skim milk instead of raw milk was used as the source of milk plasma for "remade" milks, there was no significant lipolysis in "remade" whole milks or in "remade" buttermilks, and 2. In the absence of lipolysis the curd tension reduction in "remade" buttermilks was relatively small. No complete inhibition of clotting of buttermilk by rennet was observed in any of these cases. On the other hand, in the experiments which were exactly the same in every respect except that a raw skim milk was used as the source of milk plasma for "remade" milks, our data are in complete agreement with the data obtained by Tarassuk and Palmer (3). The "remade" buttermilks in this instance, on aging at a low temperature, did not show the slightest visible coagulation on the addition of more rennet than was ordinarily sufficient to produce practically instantaneous clotting. The above "remade" buttermilks were characterized by abnormally low pH and surface tension values, suggesting the presence of free fat acids as the result of a rather extensive lipolysis. Though to a much smaller degree than in "remade" buttermilk, lipolysis was also evident in "remade" whole milks if their source of milk plasma was a raw skim milk. Further evidence of hydrolysis of fat in "remade" milks whose milk plasma was a raw skim milk is given in table 3.

It should be noted that in the present experiments, as in the experiments of Tarassuk and Palmer (3), the degree of dispersion of fat globules in synthetic creams was similar to that of natural cream as determined micro-

TABLE 1

Curd tension, pH, surface tension and composition of "remade" milk from gelatin and acid whey powder creams

Experiment No.	Product tested	Curd tension	pH	Surface tension at 20-21° C.	Fat	Solids-not-fat	Remarks
		<i>gm.</i>		<i>dynes per cm.</i>	<i>%</i>	<i>%</i>	
I	Gelatin creams						
	(a) <i>Source of milk plasma: raw skim milk</i>						
	(1) Original skim	80	6.56	53.9	0.02	9.38	(1) On aging at 7° C. for two hours.
	"Remade" whole	50	6.49	46.2	3.93	7.89	
	"Remade" skim	50	6.51	49.0	0.12	8.38	
	"Remade" butter-milk	35	6.12	38.5	0.30	8.31	
	(2) Original skim	82	(2) On aging at 7° C. for 44 hours.
	"Remade" whole	45	
	"Remade" skim	54	
	"Remade" butter-milk	0	6.05	
	(b) <i>Source of milk plasma: pasteurized skim milk</i>						
	(1) "Remade" whole	46	6.63	50.4	4.20	8.13	(1) On aging at 7° C. for two hours.
	"Remade" skim	50	6.60	53.9	0.02	8.58	
	"Remade" butter-milk	36	6.60	49.0	0.80	8.10	
	(2) "Remade" whole	40	(2) On aging at 7° C. for 44 hours.
	"Remade" skim	50	
	"Remade" butter-milk	32	6.53	
II	Whey powder cream						
	<i>Source of milk plasma: raw skim milk</i>						
	Original skim	68	6.63	53.9	0.04	9.00	On aging at 4° C. for two hours.
	"Remade" whole	46	6.56	45.5	4.00	7.62	
	"Remade" skim	55	6.66	46.2	0.15	7.95	
	"Remade" buttermilk	0	6.19	37.8	0.85	7.58	
III	<i>Source of milk plasma: pasteurized skim milk</i>						
	Original skim	40	6.56	53.9	0.03	8.92	On aging at 4° C. for two hours.
	"Remade" whole	26	6.60	49.7	4.10	7.48	
	"Remade" skim	29	6.56	53.9	0.06	7.81	
	"Remade" buttermilk	16	6.70	49.0	1.90	7.48	

scopically. The subsequent lipolysis in "remade" milks, therefore, cannot be attributed to increased surface area of milk fat as has been generally attributed in the case of homogenization of raw milk and cream. The true explanation must lie in the replacement of natural "membrane" material around fat globules. The phenomenon of hydrolysis of milk fat when an unnatural emulsifying agent is substituted for the natural fat globule adsorption "membrane" was pointed out by Tarassuk and Palmer (3). That

such treatment is conducive to lipolysis is also evident from a later report by Krukovsky and Sharp (5) on lipolysis of milk fat emulsified in pasteurized skim milk and diluted to a whole with raw skim milk. These authors chose the term "resurfacing" of fat globules to designate the presence of adsorption layer or fat globules of other composition than a natural one.

In connection with the properties of the fat globule adsorption "membrane" derived from skim milk (skim milk is used as emulsifying agent to make the synthetic cream), the lipolysis takes place in "remade" milks obtained from this cream to about the same extent as in similar milks from gelatin and acid whey powder creams. However, in spite of lipolysis, the coagulation of "remade" buttermilk from "skim milk" cream is normal and curd tension reduction is relatively only slight, as can be seen from table 2. Similar results were obtained by Tarassuk and Palmer (3) when an aqueous solution of skim milk powder was used as an emulsifying agent.

TABLE 2

Curd tension, pH, and surface tension of "remade" milks from "skim milk" and natural fat globule "membrane" creams

Experiment No.	Product tested	Curd tension	pH	Surface tension at 20-21°C.
		gm.		dynes per cm.
IV	"Skim milk" cream <i>Source of milk plasma: raw skim milk</i>			
	Original skim	64	6.60	53.9
	"Remade" whole	58	6.49	44.8
	"Remade" skim	61	6.56	49.3
	"Remade" buttermilk	54	5.88	42.0
V	"Natural fat globule 'membrane'" cream (a) <i>Source of milk plasma: raw skim milk</i>			
	Original skim	60	6.64	53.9
	"Remade" whole	39	6.54	49.0
	"Remade" skim	42	6.60	51.1
	"Remade" buttermilk	0	6.12	42.3
	(b) <i>Source of milk plasma: pasteurized skim milk</i>			
	Original skim	60	6.64	53.9
	"Remade" whole	42	6.62	50.4
	"Remade" skim	46	6.64	52.5
	"Remade" buttermilk	27	6.65	51.0
VI	Natural cream			
	Whole milk (raw)	68	6.58	49.0
	Skim milk (raw)	84	6.59	53.2
	Buttermilk from raw cream	50	6.53	44.0
	Buttermilk from pasteurized cream	48	6.60	46.9
VII	Buttermilk from natural pasteurized cream	48	6.59	47.2
	Buttermilk from natural pasteurized cream + steapsin*	0	5.58	33.6

* On the addition of steapsin to cream, the cream was aged until a distinct rancid flavor was developed, and then churned.

The data in table 2, experiment V, show also that the natural "membrane" material, once removed from the fat globules by churning no longer possesses, to the same extent, the property of protection from lipolysis. In experiment V fresh sweet cream was washed three times, each with four volumes of distilled water at 35° C. The washed cream was churned, and the buttermilk from washed cream, pervaporated to two-thirds of original volume, was used as emulsifying agent in preparation of a synthetic cream. This cream is designated as "natural fat globule 'membrane'" cream. The "remade" milks were made from this cream in the normal way using raw skim milk in (a) and pasteurized skim milk in (b) parts of experiment as the source of milk plasma. This skim milk was obtained from the same lot of whole milk as the washed cream.

For the sake of comparison, in the separate experiment VI, table 2, the typical curd tension, pH and surface tension data of buttermilks obtained by churning a natural fresh, raw cream and the same cream but pasteurized are given.

The additional and direct proof of the phenomenon of total inhibition of clotting of buttermilk by rennet due to lipolysis is demonstrated by the data of the experiment VII, table 2. The buttermilk obtained from cream to which steapsin has been added exhibited exactly the same properties in respect to non-coagulation with rennet, low pH and low surface tension values as the buttermilks from "remade" creams whose milk plasma was a raw skim milk. The fact and the extent of hydrolysis of fat in such creams is also shown by the amount of their free fatty acids; the data are given in table 3. The experiment numbers in this table correspond to the respective experiments of tables 1 and 2.

II. THE EFFECT OF CERTAIN FAT ACIDS ON COAGULATION OF MILK BY RENNET

The phenomenon of inhibition of rennet clot in buttermilks from "remade" creams in which hydrolysis of fat has taken place leads to the question as to the constituent of fat affected and the mechanism involved in the prevention of clotting of buttermilk by rennet. Theoretically, the release of fat acids on hydrolysis of milk fat should cause faster coagulation of milk or buttermilk by rennet and give higher curd tension due to the lowering of pH. And, indeed, that is what happens when fatty acids of lower molecular weight, such as caproic, are added to milk in the amount necessary to lower the pH of milk comparable to the pH of "remade" buttermilks. However, the addition to milk of higher melting point fat acids, such as lauric, myristic or palmitic, inhibits the coagulation of milk by rennet completely when the conditions in respect to the amount added (as judged by the lowering of pH of milk) and aging at low temperature are comparable to those of buttermilk from "remade" creams. The complete inhibition of clotting of milk by

TABLE 3
Free fatty acids of fat from various "remade" creams*

Experiment No.	Description of fat	Acid degree†
I	Gelatin creams	
	Original fat (before emulsification)	0.67
	(a) Fat from "remade" cream	5.05
	Source of milk plasma: raw skim milk	
	(b) Fat from "remade" cream	0.62
II	Source of milk plasma: pasteurized skim milk	
	Whey powder creams	
	Fat from "remade" cream	5.02
III	Source of milk plasma: raw skim milk	
	Whey powder creams	
	Original fat (before emulsification)	0.78
	Fat from "remade" cream	0.72
IV	Source of milk plasma: pasteurized skim milk	
	Skim milk cream	
	Original fat (before emulsification)	0.61
	Fat from "remade" cream	7.45
V	Source of milk plasma: raw skim milk	
	Natural fat globule "membrane" creams	
	Original fat (before emulsification)	0.71
	(a) Fat from "remade" cream	2.92
	Source of milk plasma: raw skim milk	
VI	(b) Fat from "remade" cream	0.75
	Source of milk plasma: pasteurized skim milk	
	Natural cream	
	Fat from raw cream	0.96
	Fat from pasteurized cream	0.67

* The "remade" creams were about 30 hours old at the time of their churning and isolation of fat for acid degree.

† ml. 1N NaOH/100 gm. fat.

these fat acids¹ is demonstrated by the data of table 4. The addition of lauric (M. Pt. 43.6° C.), myristic (M. Pt. 54° C.) and palmitic (M. Pt. 62.6°–63° C.) acids to milk necessitated melting them and adding them in a liquid state to the milk which was also warmed to the temperature of the melting point of the fat acid added. On the addition of the fat acid to milk the mixture was maintained at the melting-point temperature of the respective fat acid for an additional 20–25 minutes, with frequent vigorous stirring to assure a thorough dispersion of the acid in the milk. After this treatment the milk was cooled in ice cold water to 5–7° C. and aged at 7° C. for at least two hours before the curd tension test was made, the latter being made at 35° C.

The question naturally arises as to the mechanism by which the fat acids

¹ Fatty acids in this experiment were obtained from the following sources: Caproic—from Special Chemical Co., Waukegan, Illinois; lauric, myristic and palmitic from Eastman Kodak Co., and oleic acid was prepared by Dr. J. L. Henderson, by fractional distillation of the methyl esters of olive oil and purification by several crystallizations from acetone at –50° C. The oleic acid had an iodine number of 88.6.

under consideration inhibit the clotting of milk by rennet and thus reduce the curd tension of milk to zero. The following experimental evidence gives some insight into the phenomenon involved. It was found that on the addition of fat acids to milk in the manner described, it is essential to cool the milk and hold cold for some period of time in order to obtain a zero curd tension. One hour of aging at 7° C. seems to be the minimum aging time necessary. When the milk + 0.3 per cent of lauric acid of experiment II, table 4 was tested immediately upon cooling to 7° C. the clotting of milk by rennet was merely delayed although the curd formed was very weak and soft. The sample of the same milk aged for 1 hour at room temperature gave a normal rennet clot; while the sample aged for 2 hours at 7° C. did not show a trace of visible coagulation on the addition of more than double the amount of rennet necessary for an instantaneous clotting of the original sample of milk.

It can be seen from the data of experiment I, table 4, that the addition of CaCl_2 to milk nullifies the inhibitive effect of fat acids in rennet coagulation of milk. The presence in milk of an adequate concentration of Ca^{++} is essential for a normal clotting with rennet. It may be argued then that the inhibitive effect of fat acids on rennet coagulation is due to the formation of highly insoluble calcium salts of these acids. The necessity of cooling and aging in itself speaks against the argument of tying up calcium ions by formation of calcium salts of fat acids as the true explanation. Besides it is very unlikely that an appreciable amount of calcium salts of fat acids will be formed at the pH values experienced. The treatment of milk coupled with the amount and the kind of fat acids necessary in order to obtain a complete inhibition of rennet coagulation suggests an adsorption film of the fat acid on the colloidal complex involved in clotting of milk. The following experimental evidence bears out the film theory and the importance of the physical state of the film in rennet coagulation. As has been stated in the experimental methods the temperature of the milk used for curd tension determination was 35° C. If the milk or "remade" buttermilk, exhibiting a failure to clot because of the hydrolysis of fat or the direct addition of certain fat acids as has been described, is warmed to 50°–55° C., held at that temperature for about ten minutes and then cooled to 35° C., the normal clotting by rennet and the curd tension are restored. The temperature to which the milk has to be warmed previous to cooling to 35° C., at which temperature the rennet is added, depends entirely on the melting point of the fat acid involved in the inhibition of rennet coagulation. Thus, in the samples of milk in which a failure to clot was due to the addition of lauric acid, the warming to the temperature of 42–44° C. for about ten minutes is fully sufficient for a restoration of curd tension. The melting point of lauric acid is 43.6–44° C.

TABLE 4
Effect of addition of certain fat acids to milk on curd tension, pH and surface tension of milk

Experiment No.	Treatment of milk	Curd tension	pH	Surface tension at 20-21° C.
		<i>gm.</i>		<i>dynes per cm.</i>
I	Raw skim milk (blank)	69	6.66	53.5
	Raw skim + 0.3% of lauric acid	0	6.19	49.7
	Raw skim + 0.3% of lauric acid + 1 ml. of 5% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	75
	Raw skim + 0.3% of lauric acid + 2 ml. of 5% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	98
II	Raw skim milk (blank)	84	6.63	53.9
	Raw skim + 0.3% of lauric acid	0	6.22	49.0
	Pasteurized skim + 0.3% of lauric acid	0	6.20	49.0
	Raw skim + 0.2% of lauric acid	29	6.30	49.0
	Raw skim + 0.1% of lauric acid	80	6.42	49.0
III	Raw skim (blank)	73	6.60	53.5
	Raw skim + 0.3% of caproic acid	92	5.58	49.0
	Raw skim + 0.3% of myristic acid	0	6.07	51.8
	Raw skim + 0.3% of palmitic acid	0	6.14	51.8
	Raw skim + 0.27% of oleic acid	32	6.32	38.5

The data in table 5 illustrate the importance of physical state of fat acids involved in the inhibition of clotting of milk by rennet.

DISCUSSION

Milks and their creams whose natural fat globule adsorption "membrane" material has been replaced by the process of re-emulsification of the milk fat in aqueous solutions of gelatin, whey powder, calcium caseinate, skim milk powder or skim milk itself, exhibit a pronounced lipolysis of fat if their source of milk plasma is raw skim milk. The use of pasteurized skim milk as the source of milk plasma prevents this lipolysis. It is evident then that a normal raw skim milk has an enzyme capable of hydrolyzing milk fat and that a natural fat globule adsorption "membrane" affords some protection from this action.

In the light of the work of Herrington and Krukovsky (6) on lipase action in normal milk and our data on the acid degree of fat from natural raw cream and the same cream pasteurized, it appears that the protection against lipolysis afforded by the natural adsorption "membrane" around fat globules is not absolute. However, in our experience, the extent of lipolysis in normal raw milk² is so negligible as to be of no importance from the commercial point of view unless the enzyme is activated. It is interesting to note that all processes of activation of lipase reported in the lit-

² The so-called bitter milk of late lactation in which rancidity develops seemingly spontaneously is excluded.

erature, such as, homogenization (7), shaking (8), and temperature manipulation (9), do lead to the disruption and partial replacement or distortion of the natural adsorption layer on fat globules. This fact gives further support to the theory of protection from lipolysis by this natural adsorption layer when it is in the state of orientation on the fat globules as exists in untreated milks. The evidence that the natural fat globule adsorption "membrane" once removed from the fat globules by churning affords less protection against lipolysis suggests the partial denaturation of protein of the fat globule "membrane" as a possible explanation. The Rahn theory of denaturation of surface-active protein material in the process of churning is also supported by the recent work of Clayton (10).

That the phenomenon of curd tension reduction or total inhibition of clotting of buttermilks from "remade" creams by rennet is due largely to hydrolysis of milk fat in "remade" creams has been amply demonstrated by the differences in curd tension obtained in the presence and the absence of hydrolysis of fat in "remade" creams, as well as, by the experiments in which the same phenomenon has been produced by a direct addition of high melting point fat acids to milk or by the addition of steapsin to cream. However, even the very extensive hydrolysis of fat in cream will not lead to a complete inhibition of clotting of buttermilk by rennet in all cases as is shown by the data on "remade" buttermilk from "skim milk" cream in table 2. Since the presence of lower molecular weight fat acids would counteract the curd tension reducing effect of high melting point fat acids it seems safe to assume that the final effect on curd tension would be determined by the amount and the kind of fat acids produced in hydrolysis of fat in a particular cream. This would indicate that the material surrounding fat globules might be a factor in selective hydrolysis by the enzyme.

The clue to the mechanism of inhibition of coagulation of milk by rennet by certain fat acids is found in the fact of the partial or complete restoration of normal properties when the milk is warmed to a sufficiently high temperature to soften or melt the fat acids. On the basis of surface tension data the adsorption of the fat acids by the calcium caseinate complex of milk would be expected. The formation by fat acids of surface films consisting presumably of single layers of oriented molecules is a well-established fact. There is a considerable difference in the packing of oriented molecules in the film depending on the physical state of the fat acid. The packing of molecules of fat acid would be much greater when the acid is in a solid state. This packing of oriented molecules in the film is seemingly great enough when the acid is in a solid state to prevent the action of rennet on calcium caseinate of milk. This explanation is supported by the work of Söhngen, Wieringa and Pasveer (11) in which some experimental evidence is given that the rennin has to be adsorbed on the casein molecule in order to be effective.

TABLE 5

Effect of heat treatment on curd tension of milks whose rennet coagulation is inhibited by hydrolysis of milk fat or by direct addition of certain fat acids

Sample No.	Treatment of milk	Curd tension
		gm.
1	Raw skim (blank). Standard procedure*	81
2	Raw skim + 0.3% of lauric acid. Standard procedure	0
1(a)	Raw skim (blank). Warmed to 44° C., held at 43°-44° C. for 10 minutes and then cooled to 35° C.	79
2(a)	Raw skim + 0.3% of lauric acid. Treated as in 1(a)	71
3	"Remade" skim from gelatin cream. Standard procedure	70
4	"Remade" buttermilk from gelatin cream. Standard procedure	0
3(a)	"Remade" skim from gelatin cream. Warmed to 55° C. and held at 50-55° C. for 10 minutes	67
4(a)	"Remade" buttermilk from gelatin cream. Treated as in 3(a)	42
5	"Remade" skim from whey powder cream. Standard procedure	54
6	"Remade" buttermilk from whey powder cream. Standard procedure	0
5(a)	"Remade" skim from whey powder cream. Treated as in 3(a)	50
6(a)	"Remade" buttermilk from whey powder cream. Treated as in 3(a)	44

* Under the standard procedure rennet is added on warming the milk to 35° C.

CONCLUSIONS

1. A replacement of the natural adsorption "membrane" of the fat globules by other surface-active material in raw milk or cream promotes an extensive lipolysis of the milk fat.

2. The rennet coagulation of buttermilk obtained by churning a natural or synthetic cream in which an extensive hydrolysis of fat has taken place may be completely inhibited. The inhibition is due to the interference of high melting point fat acids with the normal action of rennet.

3. The addition of lauric, myristic or palmitic acid to milk inhibits completely the rennet coagulation if the conditions as to the amount of the acid added and the aging of the milk in cold after the addition of acid, are satisfied.

4. The normal rennet coagulation and curd tension of milk are restored if the physical state of the fat acids involved is changed from the solid to (or near to) the liquid state.

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EFFECT OF HUMIDITY ON MOISTURE CONTENT AND FORMS OF LACTOSE IN DRIED WHEY

PAUL F. SHARP AND HUGO DOOB, JR.

Department of Dairy Industry, Cornell University, Ithaca, New York

INTRODUCTION

Dried whey contains 60 to 75 per cent of anhydrous lactose in the dry matter, depending largely upon the extent of lactose fermentation prior to drying. If whey is dried by the ordinary spray or roll drying process in the manner customarily used for milk, the lactose does not crystallize but remains in the form of a syrup or glass. Whey dried in this manner is very hygroscopic. At ordinary humidities it will absorb moisture and become sticky, and the lactose will finally crystallize, forming a hard cake which must be broken up. The role of lactose in the caking of dried whey has previously been discussed and demonstrated by Troy and Sharp (4). Several patents have been issued covering various processes for inducing lactose crystallization prior to the complete drying of whey (1).

Holm and Greenbank (2) and Supplee (3) determined the moisture content of dried milk after storage at a series of relative humidities. Troy and Sharp (4) showed that a change of beta to alpha lactose occurred on holding dried milk at a high humidity and that this change was accompanied by the appearance of alpha hydrate crystals in the dried milk. The present paper reports results obtained with dried whey. The lactose content of dried whey is higher than that of dried milk and consequently the changes in the lactose are more strikingly reflected in the behavior of the product when exposed to varying humidities. In one type of non-caking dried whey a large proportion of the lactose is present as crystalline alpha hydrate, in another, as beta anhydride. Much information in regard to the properties of lactose has been gained by a study of methods of drying and the properties of dried whey.

EXPERIMENTAL

Control of humidity. Dried whey samples of 3 grams each were placed in open aluminum dishes 5 centimeters in diameter. The dishes were placed in uniform large desiccators with straight sides. In the bottom of each desiccator were placed two liters of an appropriate solution of sulphuric acid to give the desired relative humidity at 25° C. The sulphuric acid solutions were prepared according to the data given in the International Critical Tables. The dishes were weighed at the end of $\frac{1}{4}$, 1, 3, 5, 15, 21, 34, and 68 days.

Other experiments have shown that several years may be required for

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the complete adjustment of the samples to some of the humidity levels. However, for ordinary practical purposes the adjustment of the moisture content of the samples is fairly complete in one to three weeks.

Types of dried whey. The samples of dried whey studied divided themselves roughly into two groups, depending on whether alpha lactose hydrate or beta anhydride had crystallized to form an appreciable amount of solid phase.

The sample numbers followed by the letter "S" refer to samples prepared by the authors, using small scale manufacturing equipment; otherwise, the samples were from commercial products obtained from various sources.

The process of drying whey in which beta lactose crystallizes as a solid phase was developed by Lavett and is essentially as follows: Whey is first concentrated by evaporation to between 30 and 50 per cent solids. The whey is further concentrated to about 80 or 85 per cent total solids on double atmospheric rolls rotating outward from the pinch. The taffy-like mass removed by the knives drops on the surface of a second pair of double atmospheric rolls placed directly beneath the first pair but turning inward toward the pinch. The mass is continuously seeded with beta crystals adhering to the roll surface and the drying is completed on this second pair of rolls. The dry product removed by the knives is flaky and easily pulverized, and a large part of the lactose is present as beta crystals.

The following processes induce lactose crystallization as the alpha lactose hydrate:

(a) In the Simmons process whey is concentrated to between 60 and 70 per cent solids by evaporation. This concentrated syrup is drawn into crystallizing vats. The cooled mass may be mixed with a portion of whey from a preceding lot, or even with dried whey, in order to seed the mass with alpha hydrate crystals. This mixture is allowed to set for some hours, permitting a considerable amount of alpha lactose hydrate to crystallize. After this setting period the material is broken up into small particles and drying is completed by warmed air.

(b) In the Peebles and Manning process whey is concentrated to between 30 and 50 per cent solids, and sprayed into a conical dryer. The spray drying is so controlled that the whey is not completely dried. The product is then mixed and introduced into a rotating drum, together with a little moisture in the form of steam. The temperature and moisture content are so adjusted as to induce crystallization of the lactose as alpha hydrate. Drying is then completed by air.

(c) Whey is concentrated to 60 per cent solids and is then agitated in a crystallizing vat, preferably with seeding from a previous lot. After a considerable amount of lactose has crystallized and the material has thickened to a very considerable extent, drying and crystallization are completed on

rolls in a vacuum; the rolls are maintained at an interior temperature between 140° and 170° F. In this way, with the completely seeded mass applied to the roll and the temperature maintained well below the so-called inversion point of lactose, drying accompanied by crystallization of the lactose as alpha hydrate is accomplished.

(d) Whey was concentrated by various methods in small plant equipment. Crystallization of lactose as alpha hydrate occurred spontaneously or was induced by seeding. Drying was completed on trays in a drying tunnel.

Results. Figure 1 shows that the adjustment of the moisture content of small samples of dried whey placed at various relative humidities is approximately complete in one to three weeks. Two typical samples of dried whey are illustrated; in one, a considerable amount of the lactose was pres-

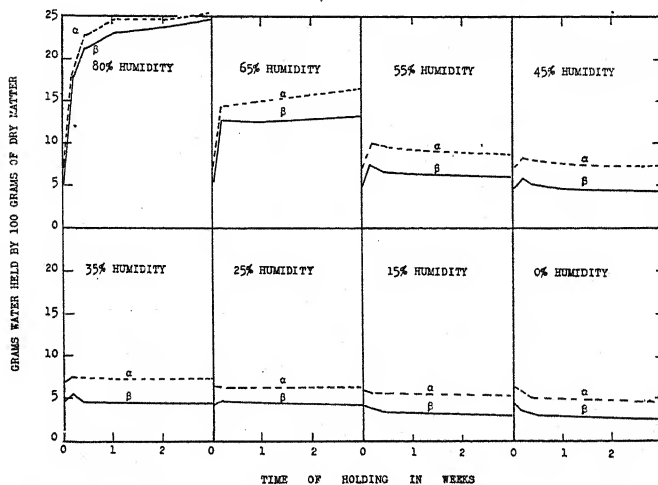


FIG. 1. Water-holding capacity of dried whey at 25° C. at various relative humidities. In one case alpha hydrate, in the other, beta anhydride is present as the solid phase.

ent as crystalline alpha hydrate, in the other, as crystalline beta anhydride. These samples were sealed by the manufacturer and were received in airtight containers. Figure 1 indicates that whey is dried commercially to the extent of being in equilibrium with a relative humidity of approximately 20 to 25 per cent at room temperature.

A number of samples of whey dried by the various methods were tested in order to determine the variations among samples. Some of the data obtained are presented in table 1. It will be observed that the samples roughly divide themselves into two groups. The samples containing beta lactose as a solid phase tended to contain less moisture when exposed to humidities below 65 per cent than samples containing alpha lactose hydrate as a solid phase exposed to the same relative humidities. This would be

TABLE 1
Grams of water held by 100 grams of dry matter in dried wheys kept at various relative humidities for three weeks at 25° C.

Sample No.	Lactose			Relative humidity at 25° C.									
	Total in solids	Proportion of		95%*	80%	65%	55%	45%	35%	25%	15%		
		Alpha	Beta										
		%	%										
		Beta lactose solid phase, concentrated, atmospheric drum dried											
28	62.8	21.0	79.0	58.0	25.2	12.7	6.6	5.3	4.8	4.5	3.5		
70	71.9	25.5	74.5	50.8	18.8	11.7	4.5	3.4	3.0	4.0	2.9		
71	69.0	18.4	81.6	61.6	24.7	14.7	6.1	4.5	4.4	4.2	3.4		
36	67.6	19.6	80.4	63.0	24.3	13.2	6.1	4.8	4.4	4.1	3.2		
Alpha lactose solid phase concentrated, seeded, then air dried													
67	69.9	92.0	8.0	62.3	24.2	16.3	8.9	7.7	7.5	6.4	5.6		
22	68.1	90.1	9.9	76.0	31.7	15.4	8.0	7.2	6.6	5.5	4.9		
26	60.1	97.3	2.7	82.5	28.6	20.6	10.5	8.7	8.1	7.0	6.2		
Alpha lactose solid phase concentrated, spray dried, air dried													
49	68.2	89.7	10.3	52.9	24.5	14.9	9.4	8.5	8.2	7.4	6.7		
73	67.7	88.4	11.6	60.3	25.0	15.0	10.0	8.6	8.2	7.6	7.0		
Alpha lactose solid phase concentrated, seeded, vacuum drum dried													
18	67.3	91.7	8.3	60.6	31.0	7.9	6.7	6.0	5.0		
28	71.6	55.0	45.0	58.4	30.1	7.5	6.4	6.1	5.2		
Alpha lactose solid phase, various methods of concentrating and air drying													
108	63.0	93.2	6.8	74.5	31.3	22.1	11.4	10.9	10.9	10.8	6.6		
168	74.5	91.4	8.6	51.0	21.2	10.4	7.1	6.4	6.2	5.6	5.0		
418	72.4	92.5	7.5	44.4	17.5	12.1	8.7	7.8	7.6	6.8	6.4		
478	70.8	90.0	10.0	48.9	20.2	12.6	8.8	8.1	7.8	7.0	6.2		
718	69.0	88.0	12.0	64.9	27.8	18.9	11.0	9.5	9.1	7.9	7.2		

* 7 to 14 days.

expected since the molecule of water which forms part of the alpha hydrate crystal is removed in the moisture determinations, and consequently in table 1 is reported as a part of the moisture content or non-dry matter content. The relative proportions of alpha and beta lactose in the samples given in table 1 represent the composition at the start of the experiment. They were determined from the initial and final (equilibrium) rotations of an extract, clarified with alcoholic mercuric chloride and decolorized with norrit. Total lactose was determined from the final rotation, sample weight and dilution (5).

Figure 2 shows the relationship between relative humidity and moisture-holding power of two typical dried wheys. Large amounts of moisture are

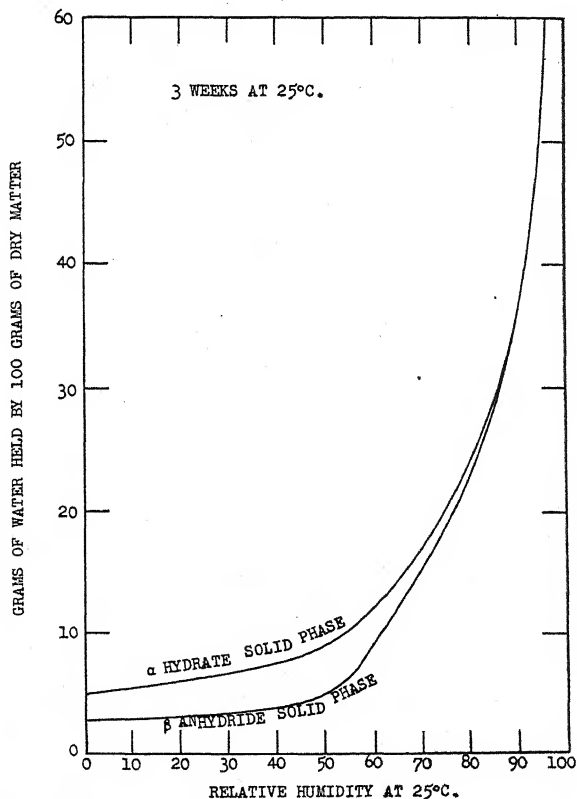


FIG. 2. Effect of relative humidity on the amount of moisture present in dried whey.

not absorbed by stabilized dried whey until the relative humidity exceeds 50 per cent. Table 2 gives the moisture content of a series of additional samples of dried whey when held at different relative humidities for one week and includes the humidity range between 80 and 95 per cent. Samples containing beta lactose as a solid phase contained less water than did sam-

TABLE 2

Grams of water associated with 100 grams of dry matter in wheys held at various relative humidities for one week at 25° C.

Relative humidity	α hydrate solid phase					β lactose solid phase	
	α seeded vac. roll dried		α seeded tray dried			Atm. drum dried	
	A	B	Old sample	Fresh samples		Flakes ground	From flakes
			C	D	E	F	G
%	gm.	gm.	gm.	gm.	gm.	gm.	gm.
95	60.6	58.4	52.2	59.1	52.3	42.3	43.3
90	47.1	45.9	35.8	44.1	42.4	31.7	32.5
85	37.4	35.9	27.4	34.3	33.5	24.7	25.9
80	30.0	30.5	23.5	29.4	28.3	20.6	21.0
55	8.4	8.0	8.5	9.0	8.8	6.2	6.2
35	6.8	6.6	6.9	7.1	6.9	4.4	4.4
25	6.0	6.1	6.2	6.1	6.1	5.1	5.2
15	5.1	5.3	5.9	5.7	5.7	4.3	4.3
0	4.8	4.0	5.0	4.5	4.5	3.1	3.2
0*	3.9	2.6	4.8	3.8	3.8	2.6	2.7

* Over concentrated sulfuric at end of 62 days.

ples containing alpha lactose hydrate as a solid phase. Grinding the sample had little or no effect on moisture absorption. After standing a number of weeks at humidities of 65 per cent and higher there is little difference in moisture content of wheys dried by various methods.

The effect was studied of maintaining the relative humidity constant at 55 per cent but varying the temperature. Results obtained with a series of typical samples are given in table 3. Temperature does not exert a very marked effect upon the equilibrium moisture content of the samples when exposed to a constant humidity. Apparently it does affect the rate of moisture equilibration. The samples maintained at 45° C. had darkened very much by the time the experiment was concluded.

Effect of relative humidity upon the proportions of alpha and beta lactose. The lactose in the various samples of dried whey as freshly prepared by the procedures discussed in this paper is present largely in the crystalline form either as alpha hydrate or as beta anhydride, but a portion, unable to crystallize because of the speed of drying, remains in the glassy state as a mixture of uncrystallized alpha lactose and uncrystallized beta lactose. If the moisture content of the dried whey is maintained at a low value, the lactose remains for long periods of time in an unchanging condition. But if the dried whey is permitted to absorb moisture, as when held at humidities between 30 and 50 per cent and the glass is sufficiently diluted to permit the movement of molecules, the particular solid form of lactose present continues to crystallize slowly, be it alpha hydrate or beta anhydride. Mutarotation takes place simultaneously to compensate for the corresponding

TABLE 3

Grams of water held by 100 grams of dry matter in dried whey after various periods of holding at 55 per cent relative humidity at 2, 25, and 45° C.

Number of days held	Beta type				Alpha type									
	28	70	71	36	67	22	26	49	73	10S	16S	41S	47S	71S
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
45° C. (113° F.)														
0	4.5	3.6	4.5	4.3	6.7	5.4	7.1	7.6	10.8	7.8	5.1	7.1	7.1	8.1
1	7.4	4.7	7.1	6.9	9.7	8.7	11.5	10.6	10.9	12.9	7.3	8.7	9.0	11.6
3	7.4	4.5	6.7	6.6	9.2	8.5	11.2	10.2	10.8	12.5	7.2	8.7	8.8	11.5
7	6.4	4.2	6.2	6.2	8.7	8.0	11.0	9.7	10.0	11.7	7.0	8.4	8.5	11.2
14	5.9	4.4	5.8	5.9	8.4	7.8	10.2	9.3	9.6	11.5	7.0	8.3	8.3	10.9
21	5.5	4.6	5.6	5.6	8.3	7.7	9.8	8.8	9.2	10.5	6.9	8.3	8.3	10.7
25° C. (77° F.)														
0	4.5	3.6	4.5	4.3	6.7	5.4	7.1	7.6	10.8	7.8	5.1	7.1	7.1	8.1
1	7.8	5.2	7.3	7.4	9.9	9.0	11.1	10.6	10.4	12.2	7.5	9.1	9.6	11.6
3	7.4	4.8	7.2	6.9	9.5	8.8	11.0	10.3	10.4	12.1	7.3	8.9	9.3	11.4
7	7.0	4.5	6.4	6.5	9.2	8.0	10.7	10.0	10.1	11.6	7.1	8.7	8.9	11.1
14	6.7	4.4	6.2	6.2	9.0	8.0	10.5	9.5	10.0	11.4	7.0	8.6	8.7	11.0
21	6.6	4.5	6.1	6.1	8.9	8.0	10.5	9.4	10.0	11.4	7.1	8.7	8.8	11.0
2° C. (34° F.)														
0	4.5	3.6	4.5	4.3	6.7	5.4	7.1	7.6	10.8	7.8	5.1	7.1	7.1	8.1
1	8.0	6.1	7.2	7.7	8.4	7.9	9.4	9.4	9.4	10.0	7.2	8.3	8.7	10.0
3	8.2	7.2	7.6	8.0	8.9	8.5	10.0	9.7	9.4	11.0	7.4	8.5	9.1	10.6
7	7.5	5.5	6.9	7.2	9.1	8.4	10.2	9.7	9.4	11.1	7.2	8.5	9.1	10.7
14	7.2	4.6	6.4	6.7	9.0	8.4	10.3	9.6	9.4	11.1	7.2	8.5	9.1	10.6
21	7.0	4.5	6.2	6.5	9.0	8.5	10.3	9.6	9.4	11.1	7.2	8.5	9.1	10.5

depletion in the glass, a disturbance of equilibrium. Evidence that this change has occurred is found in the altered relative proportions of beta and alpha lactose as well as in the absorption and subsequent liberation of moisture at constant relative humidity.

TABLE 4

Percentage of total lactose in the alpha form after holding 3 weeks at 25° C. at various relative humidities

Sample No.	Relative humidity at 25° C.						
	95*	80	65	55	45	35	25
	%	%	%	%	%	%	%
Beta solid phase, concentrated, atmospheric drum dried							
28	86.5	95.4	41.3	7.0	7.7	9.1	14.6
70	86.0	95.1	93.8	12.7	11.5	12.9	19.6
71	84.3	94.3	81.2	7.2	7.2	10.3	14.0
36	84.8	92.4	46.0	6.0	9.4	8.0	13.3
Alpha solid phase, concentrated, seeded, air dried							
67	72.9†	88.8†	92.0	94.4	93.4	90.5	90.5
22	79.0	93.5	95.5	93.2	90.5	88.2	87.0
26	75.8	83.3†	92.9	97.2	95.9	91.9	90.5
Alpha solid phase, concentrated, spray dried, air dried							
49	85.3	92.4	93.4	93.4	91.0	88.5	88.9
73	82.4	89.1	91.8	91.0	84.5	87.7	88.8
Alpha solid phase, various methods of concentrating and air drying							
10S	71.4	91.4†	92.0	98.2	93.0	92.1	87.4
16S	86.0	91.6†	93.9	94.9	90.9	91.5	87.2
41S	89.4	94.6	92.6	91.7	90.9	91.6	87.9
47S	84.2	94.6	93.2	90.6	90.4	87.7	86.1
71S	85.2	92.5	91.3	89.0	90.9	88.4	88.0

* Held 2 weeks.

† Mold.

Table 4 gives in per cent that fraction of the total lactose existing as the alpha form in the samples, at the end of the experiment presented in table 1. Table 4 shows that at about 50 per cent relative humidity and 25° C. additional alpha hydrate crystallized in those samples containing alpha hydrate as the solid phase, and additional beta anhydrate crystallized in those samples containing beta lactose as solid phase. The proportions of alpha and beta lactose in the dried whey after holding at 50 per cent humidity were altered. They were altered in a direction corresponding either to crystallization of alpha hydrate, or of beta anhydride, the direction depending on the solid form present. This is indicated by a comparison with the original product or the product held at 25 per cent humidity for the same length of time.

The original analyses for some of the samples do not agree very well with those obtained from corresponding aliquots held at 25 per cent relative humidity. The direction of the discrepancy suggests that some of the samples had accidentally become exposed to moisture in the interval between the original analysis and placing aliquots in the humidity jars.

At higher humidities of 65 and 80 the dilution of the dried whey containing crystals of the beta type was such as to permit alpha lactose hydrate crystals to form. Since alpha lactose hydrate is far less soluble at 25° C. than is beta lactose, conversion of the solid beta lactose anhydride proceeded, through solution, to alpha lactose hydrate. At 95 per cent humidity the amount of moisture held by the whey was so great that an appreciable amount of lactose dissolved in the water. Therefore, the amount in the form of alpha lactose decreased again because crystalline alpha hydrate dissolved to form a relatively large amount of equilibrium solution. Table 5 shows that at 55 per cent relative humidity additional crystallization of lactose occurred more rapidly the higher the temperature, even though the results may appear to be a little uncertain at 45° C. because of difficulty in

TABLE 5

Percentages of total lactose in the alpha form after holding for 3 weeks at 55 per cent relative humidity at various temperatures

Sample No.	Relative humidity 55 per cent		
	2° C. 34° F.	25° C. 77° F.	45° C. 113° F.
	%	%	%
Beta solid phase, concentrated, atmospheric drum dried			
28	11.0	7.0	5.8
70	15.4	12.7	6.4
71	10.0	7.2	4.4
36	11.4	6.0	6.8
Alpha solid phase, concentrated, seeded, air dried			
67	91.3	94.4	92.4
22	88.4	93.2	96.8
26	93.1	97.2	96.2
Alpha solid phase, concentrated, spray dried, air dried			
49	89.2	93.4	95.5
73	86.8	91.0	95.6
Alpha solid phase, various methods of concentrating and air drying			
10S	91.9	98.2	99.6
16S	90.6	94.9	92.4
41S	91.1	91.7	94.6
47S	88.6	90.6	95.0
71S	87.0	89.0	92.4

analyzing some of the dark products formed at this temperature. Faster crystallization at higher temperatures occurred in both alpha and beta lactose wheys. This variation in crystallization accounts for some of the variations in moisture content of the whey in table 3.

When either dried whey or dried milk containing lactose in the form of glass is exposed to a relative humidity in the neighborhood of 50 per cent, the product at first takes up moisture to dilute the glass, and then as lactose crystallizes and the vapor tension increases moisture is released again. Consequently, there is observed an increase followed by a decrease in moisture content of the samples (3, 4). Table 3 shows that this change occurs more rapidly at higher temperatures, and table 5 presents the evidence of crystallization as reflected by the change in relative proportions of the forms of lactose.

Effect of amount of lactose in the dry matter. In the course of this work a considerable number of samples containing varying percentages of lactose in the dry matter were allowed to come to equilibrium with atmospheres of several constant relative humidities. Figure 3 shows that the lower the percentage of lactose in the dry matter, the higher the moisture content of the dried whey when exposed to an atmosphere of constant relative humidity. This figure also indicates that differences between moisture contents of dried wheys exposed to constant relative humidity are greater, the higher the relative humidity. At lower humidities samples containing beta lactose as a solid phase arrange themselves into one group, and those containing alpha lactose hydrate as a solid phase arrange themselves into another group having higher moisture content. This difference between the two types of samples is maintained up to relative humidity in the neighborhood of 65 per cent. Above this relative humidity there is only one group for the reason that at the high relative humidities the solid beta lactose disappears and is converted to solid alpha lactose hydrate. Thus all samples are very much alike in solid lactose phase.

Commercially, the main variation in lactose content of the dry matter results from varying degrees of fermentation of the sugar prior to drying. Wheys produced by mineral acid precipitation or rennet coagulation of the casein in fresh skim milk tend to be relatively high in lactose whereas cheese wheys tend to be low because of the fermentation of the lactose prior to drying. Lactic acid is the main fermentation product and we would expect the hygroscopic properties of such dried whey to be more pronounced because one molecule of osmotically active lactose would be converted into four molecules of osmotically active lactic acid. This probably accounts for the greater hygroscopicity of the dried wheys containing the lower percentages of lactose in the dry matter.

SUMMARY

1. Dried wheys in which crystalline beta lactose is present as a solid phase contain less water when in equilibrium with an atmosphere of con-

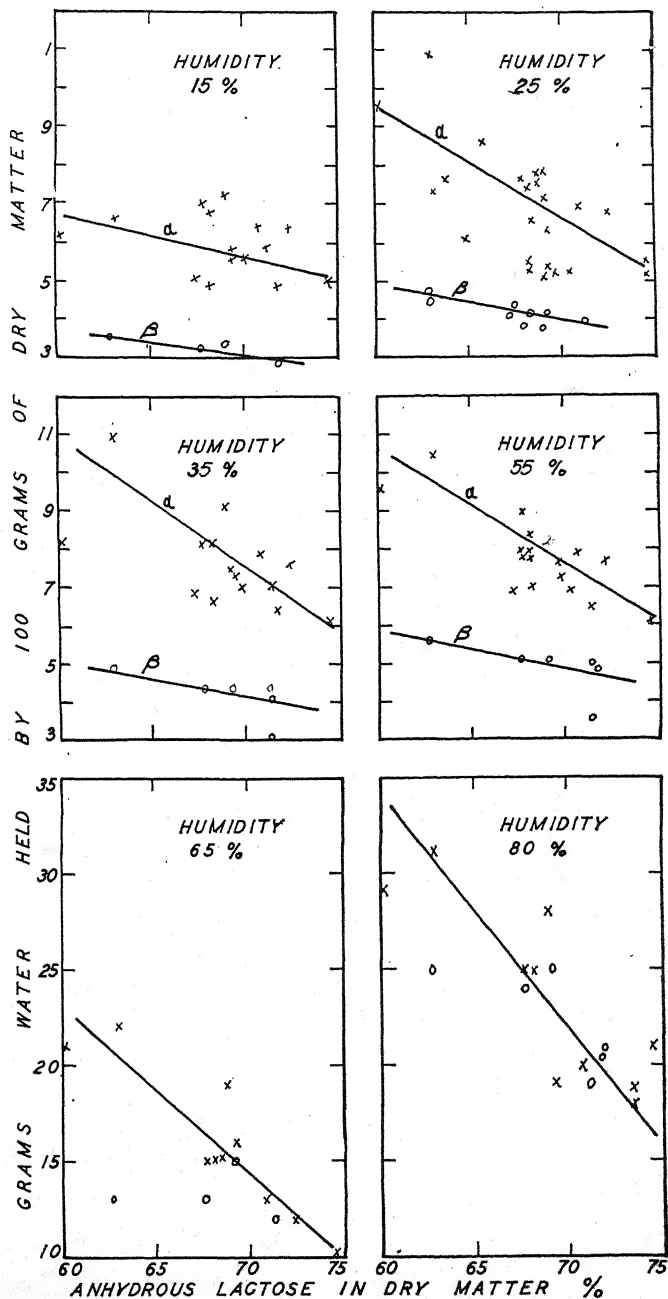


FIG. 3. The effect of percentage of lactose in the dry matter upon the moisture content of dried whey when exposed to various atmospheres of constant relative humidity at 25° C.

stant relative humidity below 65 per cent than do dried wheys in which alpha hydrate is the solid phase. The difference is largely accounted for by the molecule of water of crystallization present in the alpha hydrate crystals.

2. Stabilized dried wheys do not absorb excessive amounts of water until the relative humidity exceeds 40-50 per cent.

3. Temperature exerts no marked effect upon the equilibrium moisture content of samples exposed to constant relative humidity. The equilibrium is attained more rapidly at the higher temperatures.

4. If an appreciable amount of lactose in the glass state is present at relative humidities between 30 to 50 per cent the whey will first absorb and then reject water. This process is accompanied by the crystallization of the solid form of lactose present. In this way crystallization of beta lactose at room temperatures may occur.

5. At relative humidities of 65 per cent and above the crystalline beta lactose in dried wheys undergoes conversion to crystalline alpha hydrate.

6. The lower the percentage of lactose in the dry matter the greater the equilibrium moisture content of the dried whey at constant relative humidity.

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THE EFFECT OF CURD TENSION OF MILK ON THE UTILIZATION OF ADDED VITAMIN D

W. E. KRAUSS, T. S. SUTTON, L. H. BURGWALD,
R. G. WASHBURN AND R. M. BETHKE

*Departments of Dairy and Animal Industry, Ohio Agricultural Experiment Station,
Wooster, Ohio*

The fortification of milk with vitamin D concentrates has been practiced for some time. At first no question arose as to the possibility of the effectiveness of the added vitamin D varying with the type of milk fortified since practically all milk so treated was of the ordinary pasteurized variety. After the introduction of soft curd and homogenized milk and the presentation of some evidence that digestibility or calcium assimilation or both might be favorably affected by such processing (1-5), the effectiveness of vitamin D additions to such milk seemed worthy of consideration.

A supply of mixed milk was divided into three portions and prepared as follows: 1, untreated; 2, homogenized at 2500 pounds pressure and; 3, mineral modified (6, 7).¹ All three samples were then pasteurized at 143° F. for 30 minutes. A batch of natural soft curd milk was collected from selected cows in the Ohio State University herd and similarly pasteurized. Similar batches of milks were prepared at weekly intervals.

After each batch of milk was prepared the curd tension was determined by the tentative method adopted by the Committee on Curd Tension Measurements at the June, 1938, meeting of the American Dairy Science Association. Each batch was then fortified to the extent of 400 U.S.P. units of D per quart by adding a commercial vitamin D concentrate (Cream Vitex).

The samples thus prepared were fed to rats according to the official method for determining vitamin D by the line-test procedure and also by the prophylactic procedure which uses bone ash as a criterion.

RESULTS

The curd tension measurements are summarized in table 1. Both surface and below surface readings were made but only the maximum or surface measurement is recorded, in keeping with the Curd Tension Committee's recommendation. Each value listed is the average of two or more readings. It will be seen from table 1 that the curd tension variation was considerable, from 0.0 grams in the mineral modified milk to 54.9 grams in the normal pasteurized milk. It is also interesting to note that homogenization at 2500 pounds pressure reduced the curd tension by 61.5 per cent.

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¹ Base-exchange method; prepared through the courtesy of M. and R. Dietetic Laboratories, Columbus, Ohio.

TABLE 1

Curd tension of normal pasteurized milk, homogenized milk, mineral modified soft-curd milk, and natural soft-curd milk

Batch	Normal pas- teurized milk	Homogenized milk	Mineral modi- fied soft curd milk	Natural soft curd milk
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
1	51.5	21.0	0	35.5
2	43.5	15.0	0	46.0
3	50.0	27.0	0	29.0
4	62.5	27.0	0	35.0
5	69.0	22.0	0	34.5
6	56.5	17.0	0	25.5
7	50.0	23.5	0	32.0
Av.	54.9	21.8	0	33.9

In the curative trial (line test procedure), the results of which are reported in table 2, 6.0 cc. of each kind of milk were fed over a period of 3 days. The rats were slaughtered on the eighth day, they having had meanwhile free access to the basal rickets-producing diet. One group of rats received 26 milligrams of reference cod liver oil (2.5 units), an amount of vitamin D calculated to equal that in 6.0 cc. of milk. That the responses from the milks were greater than from the reference oil may have been due to the fact that the milks contained not only the added 400 units per quart but the vitamin D originally present, even though this amount was not sufficient to produce a response when 6.0 cc. each of the normal unfortified and normal soft curd unfortified milks were fed.

From the healing responses obtained when equal amounts of the various fortified milks were fed (table 2) it is apparent that there was no difference in the effectiveness of the added vitamin D. The slight differences in the average line test values are too small to be of significance.

TABLE 2

Comparison of line test responses (healing) obtained by fortifying different kinds of milk with 400 U.S.P. units of vitamin D per quart

Material fed	Total amount fed	No. of rats	Line test* response
Normal milk, fortified	6.0 cc	10	1.70
Homogenized milk, fortified	6.0 cc.	10	1.60
Mineral modified milk, fortified	6.0 cc.	9	1.72
Natural soft curd milk, fortified	6.0 cc.	9	1.78
Reference cod liver oil	2.5 U.S.P. units	10	1.45
Normal milk, unfortified	6.0 cc.	5	0.0
Normal soft curd milk, unfortified	6.0 cc.	3	0.0

* The numerical line test responses were obtained by assigning the following values to degrees of healing:

- = 0.0	++ = 2.0
+ - = 0.5	+++ = 2.5
+ = 1.0	+++ = 3.0
++ = 1.5	

In the prophylactic trial the rats in all but two groups were fed 2.7 cc. daily for four weeks of one of the fortified milks while having free access to the basal rickets-producing diet. The rats in one of the remaining groups each received daily 1.1 U.S.P. units of vitamin D from reference cod liver oil. This amount of vitamin D was calculated to be equivalent to the amount contained in 2.7 cc. of 400-unit milk. The final group of rats received only the basal diet.

At the end of 4 weeks the rats were killed. The femurs were removed and the bone ash determined on a fat free, moisture free basis.

TABLE 3

Comparison of calcification (bone ash) obtained by fortifying different kinds of milk with 400 U.S.P. units of vitamin D per quart (4-week prophylactic trial)

Material fed	Amount fed daily	No. of rats	Gain in weight	Bone ash
			gm.	%
Normal milk, fortified	2.7 cc.	10	41.5	46.17
Homogenized milk, fortified	2.7 cc.	10	41.3	46.37
Mineral modified milk, fortified	2.7 cc.	10	41.3	45.68
Natural soft curd milk, fortified	2.7 cc.	10	39.6	45.99
Reference cod liver oil	1.1 U.S.P. units	9	18.4	43.02
Basal diet only	ad lib	7	19.1	27.87

The results of the prophylactic trial (table 3) substantiate those obtained by the curative procedure in that no significant differences were found between the bone ash values of the groups fed the fortified milks.

DISCUSSION

In keeping with previous evidence, the data in table 1 demonstrate the curd tension-reducing effect of mineral modifying (base-exchanging) and homogenizing milk. If certain beneficial effects, such as greater digestibility, lower stomach-emptying time and increased efficiency of calcium utilization result because of such processing, as has been claimed, it might be inferred that each individual constituent was likewise benefited. That added vitamin D showed no difference in utilization may have been due to the fact that such addition did not become an intimate part of the colloid but remained a separate phase.

Although the vitamin D additions were made after the milks had been processed, a survey of a number of large plants showed that as many plants made the vitamin D addition after homogenizing as before. In preparing mineral-modified milk the usual procedure is to process over the zeolite bed before the vitamin D addition is made. In the case of natural soft curd milk the addition would, of course, be made as in other unprocessed milks

It would seem, therefore, that no claim can legitimately be made as to any merit for fortifying soft curd milks with vitamin D greater than that existing for similar additions to milk of normal curd tension.

It might be presumed that the curative trial was a measure of vitamin D *per se* and that the prophylactic trial measured not only vitamin D but calcification. It is known that lactose improves calcium utilization (8) and the higher bone ash values in the fortified milk groups than in the reference oil groups are probably accounted for by this plus the effect of the vitamin D originally present. Furthermore, a normal bone ash can be obtained by feeding an adequate amount of vitamin D in oil. This would seem to obviate the argument that in the prophylactic trial even though the mineral modified milk contained about 20 per cent less calcium than the other milks (6) calcification was just as great and therefore the calcium was more efficiently used.

SUMMARY AND CONCLUSIONS

Normal milk, homogenized milk and mineral modified soft curd milk, all from the same source, and natural soft curd milk were pasteurized and fortified to the extent of 400 U.S.P. units of vitamin D per quart. These milks varied in curd tension from 0.0 grams for the mineral modified milk to 54.9 grams for the normal milk. Bioassays of these fortified milks by the line test procedure resulted in the same degree of healing when equal amounts of each were fed. In a prophylactic trial equal intakes of the fortified milks resulted in almost identical bone ash values.

It is concluded that the effectiveness of added vitamin D is not influenced by the curd tension of milk.

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EFFECTS WHICH SELECTION OF DAMS MAY HAVE ON SIRE INDEXES*

JAY L. LUSH, H. W. NORTON, III, AND FLOYD ARNOLD

INTRODUCTION

Several studies (1, 2, 3, 4, 5, 8) have shown that when the mates of a bull are divided into a high group and a low group on the basis of their own records, the average difference between the daughters of the two groups is less than half the difference between the two groups of dams. For example in Edwards' study (3) where the mates of each of 23 bulls were divided into a high half and a low half on the basis of the mate's own milk record, the low half averaged 7,513 and the high half 10,369, a difference of 2,856 pounds. But the daughters of these two groups averaged 7,835 and 8,427, a difference of only 592 pounds, which is barely one-fifth the difference between the two groups of dams.

If offspring were always mid-way between the phenotypes of their parents regardless of how the parents were selected, or if the offspring deviated individually from that only in a random way, the average difference between two groups of daughters by the same sire would tend to be half as large as the average difference between their dams (9). It is clear that the difference between the daughters is not this large when the dams are separated into high and low groups on the basis of the very same past records which are then used to represent those dams. What does this reveal about the inheritance of differences in milk and fat production? Does it disprove, as some have inferred, the validity of the widely used sire index which sets the breeding value of the sire as equal to twice the average of his daughters minus the average of his mates? What precautions should be taken to discount the effects of selection of dams when using indexes or other data to guide breeding choices in an actual dairy population?

In a qualitative way the answers to those questions are partly known already from the simple statistical principles for estimating a cow's real ability¹ from records of her past production. That a cow's record and her real ability are not always identical is well known from the fact that the intra-herd correlation between different single records made by the same cow, when standardized for age and times milked per day, has in most studies been something of the order of +.3 to +.4, rarely going as high as

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¹ By "real ability" is meant what the cow is most apt to produce in any future lactation when she is kept under conditions *intended to be* the same as those under which the past record was made.

+ .5 except in herds where the data extended over a time long enough to permit trends in management or in the genetic level of the herd to become important.

In unselected populations each cow is as likely to have had better-than-average environment as she is to have had worse-than-average environment. Therefore, in unselected populations the discrepancies between record and real ability are random. The plus discrepancies tend to cancel the minus ones and the average record of that population tends to equal (with some sampling error, of course, which may be large where the number of cows is small) the average ability of those cows under the average environmental conditions prevailing in that population.

But when a group of cows are selected because their past records were high, one tends to get not only cows which were above average in real ability but also cows which during those past lactations were exposed to better-than-average individual environment. If such selected cows are then kept for another lactation, whatever superiority in their former records was due to their superiority in real ability will tend to appear again but the cows will be exposed to a fresh sample of intra-herd variations in environment. So far as these environmental variations are temporary and random from lactation to lactation for the same cow, they are as likely to be minus as plus in the next lactation. Therefore, the average of the future records of a selected group of cows tends (with sampling errors, of course) to equal the average of their real abilities, but to be lower than the average of the records on which they were selected.

Similarly, when a group of cows is selected because their past records were low, the selection is for a result which may have been caused either by poor ability of the cow or by her having been subject to worse-than-average environment or by both. The average real ability¹ (the average future records) of such a group will not be as low as the average of the records on which they were put in this low group.

Thus in the example cited from Edwards, the "high" cows and the "low" cows differed by 2,856 in the average of the records on which they were divided. But if in his data the repeatability was +.5 (the fraction to be used here would be higher than the usual repeatability of single records, because he used averages where a cow had more than one lactation), then the most probable difference between the real abilities of his two groups of cows would have been 1,428 and the expected difference between the two groups of daughters would have been 714, which is not vastly different from the actual 592. An r of .455, which is well in line with usual values, would have made the expected difference exactly what the actual difference was.

If the repeatability of single records in these published studies was approximately of the usual size, +.3 to +.4, most of the bias between the

sire indexes calculated on the two groups disappears when the proper correction is made for the effects which selection had in making records and real abilities of dams differ systematically; *i.e.*, for the regression of future records on selected records. However, since the actual repeatability of single records was not measured in the studies cited, it is not clear whether imperfect repeatability is the whole explanation for the daughters differing so much less than half as much as their selected dams did. The investigations reported in the present paper were undertaken to see whether still other circumstances need to be considered when using sire indexes for bulls mated to selected groups of dams.

There were two separate investigations nearly alike in procedure. The first was made by Jay L. Lush and Floyd Arnold and bits of the findings were published briefly in an abstract (7) and a press article (6). The other and more extensive study was made by H. W. Norton, III.²

ANALYSIS OF DATA

The First Study

These data were the 676 daughter-dam comparisons used in proving 103 sires in Iowa Dairy Herd Improvement Associations prior to January 1, 1937. All records were age-corrected, using the Bureau of Dairy Industry factors. Where the bull's mate had only one record the data for her and her daughter were discarded. The mates of each bull were then divided into a high half and a low half, solely on the basis of the *first* record of each cow. All the *later* records of each cow were then averaged into a single figure which was used in all subsequent computations of "later records." Thus each mate had equal weight in the average of later records, whether she had only one or several records after her first. If a bull had an even number of mates with two or more records each, all were used. If he had an odd number of such mates, the one whose first record was median in size was discarded and of course her daughter was discarded with her. This was done so that each sire would have exactly as many daughters and mates in the high group as he did in the low group. Therefore, differences in herd averages or in merit of the various sires could not affect the differences between high and low groups. If a mate had more than one daughter she was used again as many times as she had daughters.

Table 1 shows how the data were arranged for computation. Oakgrove Foxy was mated to eight cows which each had two or more records, but two of these cows had two daughters each, thus making ten daughter-dam comparisons available. The five mates with high first records are placed on the

² We are indebted to those in charge of dairy herd improvement association work in Iowa and to the Holstein-Friesian Association of America for making available in convenient form the daughter-dam comparisons on which these investigations were based, and to the American Dairy Cattle Club for assistance in the second study.

TABLE 1

Sample of data on fat production showing division into "high" and "low" groups

Sire which was being proved	High mate's records			Daughter's records		Low mate's records			Daughter's records	
	First record (X)	Later records		No.	Average (Z)	First record (X)	Later records		No.	Average (Z)
		No.	Average (Y)				No.	Average (Y)		
Oakgrove Foxy	417	3	436	3	420	270	2	366	3	410
	412	1	328	2	461	322	4	401	2	370
	372	5	365	2	476	323	3	367	1	442
	372	5	365	1	300	281	1	343	1	421
	323	2	380	1	330	281	1	343	1	261
Victory Flash	453	2	483	2	430	447	2	357	1	394
	476	1	494	1	397	326	2	458	1	438
	546	1	426	1	382	423	1	333	1	368

Averages (338 items in each column)	440.4		407.8		393.4	338.3		364.2		379.3

$$\text{Repeatability of differences in single records} = \frac{407.8 - 364.2}{440.4 - 338.3} = .43$$

$$\text{Heritability of differences in single records} = \frac{2(393.4 - 379.3)}{440.4 - 338.3} = .28$$

$$\text{Heritability of permanent differences between cows} = \frac{2(393.4 - 379.3)}{407.8 - 364.2} = .65$$

left while the five with the low first records are on the right. The mate's first record (X), the average of all the mate's later records (Y), and the average records of her daughter (Z) are shown in the same line. The rest of the study concerns the average values of X, Y, and Z for the high and for the low groups. Figure 1 shows graphically for both studies the values obtained for X, Y, and Z and the regression of Y and Z toward the average of X.

Table 2 shows the results obtained when the milk yields for the same cows were treated similarly. Naturally the distribution of the cows was not quite identical in tables 1 and 2, since fat percentage varied enough that some cows whose first fat records placed them in the high half for fat had first milk records which placed them in the low half for milk and an equal number whose first fat records were in the low half had first milk records which were in the high half.

The ratio of the Y difference to the X difference, .43 for fat and .48 for milk, shows what fraction of the variance among first records of cows mated to the same bull was due to permanent differences between the cows which made those records. The rest of the variance was caused by temporary environmental forces and circumstances which were different in later lacta-

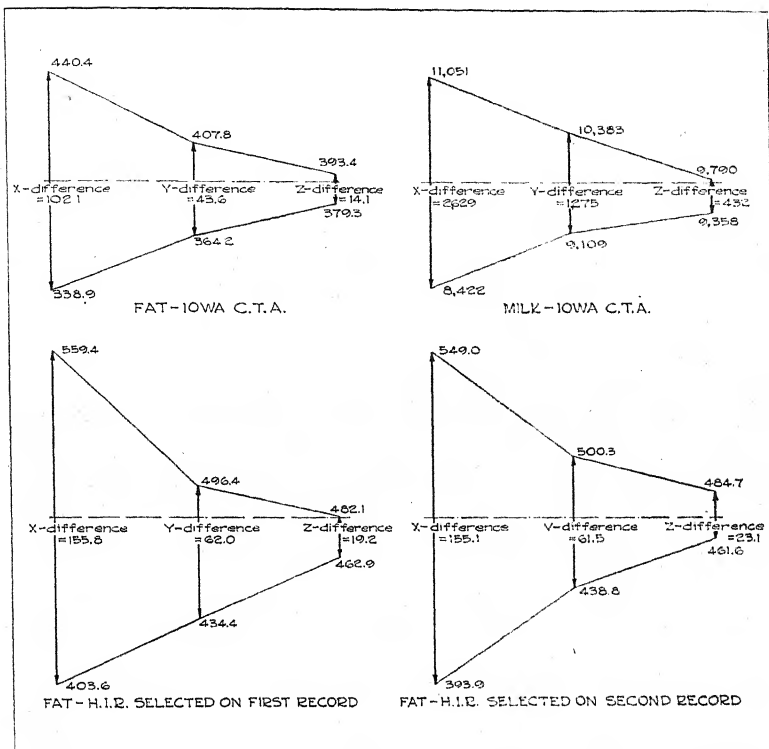


Fig. 1. Regression of later records and daughter's records from dam's selected record toward the herd average. Each horizontal line marks a level midway between the selected high records and the selected low records. The ratio of the Y-differences to the X-differences is the average repeatability of differences in the selected single records. Twice the Z-differences divided by the X-differences shows the average heritability of differences in the selected single records.

That the regression of the future fat records down from the selected high records is slightly greater than the corresponding regression up from the selected low records is interpreted as showing that some of the cows with low first records were culled before they could complete a second record, although the possibility of this being caused by a bias in the age-correction factors is not wholly excluded. That the daughters' fat records averaged below the first records of their dams and just about equal to or a little above the later records of their dams can hardly be blamed on any bias in the age correction factors, but it is in accord with the hypothesis that these dams are the survivors of some distinct selection practiced after their first records were made and that bulls bred to them were on the average just about equal to these cows in breeding value,—a bit better in the H.I.R. data.

tions. Because temporary circumstances had so much effect on the size of each record the later records (Y) regressed far toward the herd average. The real abilities of the cows differed only 43 (48 for milk) per cent as much as their records did.

TABLE 2

Average milk yields when data were divided according to size of the mate's first milk record

	Low group	High group	Difference
Mate's first record	8,422	11,051	2,629
Mate's later records	9,109	10,383	1,274
Daughter's records	9,358	9,790	432
$\frac{1274}{2629} = .48$	$\frac{2 \times 432}{2629} = .33$	$\frac{2 \times 432}{1274} = .68$	

In terms of correlation, the ratio of the Y differences to the X differences approximates the intra-herd "repeatability" of single records, *i.e.*, the correlation between single records made by the same cow.³ This repeatability coefficient describes the average condition within these populations, each of which consisted of cows mated to the same bull in an Iowa D.H.I.A. herd. Lower repeatability would be expected in populations where the cows were more uniform in their real ability or where individual environment varied more widely and irregularly from one lactation to another. Repeatability would be higher in populations within which the cows were more diverse in their real abilities or where intra-herd environmental conditions of all kinds were more rigidly equalized than here. Since each population in this study pertained to only one bull and included only those mates which had at least one tested daughter and two records of their own, and since there is little mixing of breeds in Iowa dairy herds, each such population is probably just a shade more uniform than a truly random sample taken from a whole breed but kept in the same herd would be. Cows leaving the herd before they finished two lactations could not appear here and such cows would include more than a fair share of the lowest producers. The group of cows mated to one bull would in many cases contain two or more which were half or three-quarter sisters by descent from preceding sires.

If the dam's own real ability were identical with her breeding value (which is the same thing as supposing that all differences between permanent abilities are hereditary and that the actual effect of each gene—that is, the phenotypic change produced by substituting it for its allele—is the same in every kind of genotype as it actually averages in that population), then the two groups of daughters by the same sire would tend to average half as far apart as their dams. Actually the daughters do not differ that

³ Strictly speaking, it is an approximation to the regression instead of the correlation but where the standard deviations of first records and of second or later records are equal, as was approximately true in these data, the regression of future records on single records and the correlation between single records are identical. Estimating a regression by this high-half-versus-low-half method is not quite as efficient at getting out of the data all the information they contain as is the least squares method, but it is not consistently biased in either direction and may at times be simpler to compute and to explain.

much. Half the difference between the Y values is 21.8 pounds of fat and 637 pounds of milk, whereas the actual differences between the Z values are but 14.1 pounds and 432 pounds, or about two-thirds as large as they would be if all differences between the real abilities of the dams were additively genetic.

The other third of the permanent differences in ability can have been due to one or more of three things, quite diverse in principle but not separable from each other in the present data. First, some of the permanent differences between the dams may have been caused by environmental peculiarities which were permanent throughout the lifetime of each dam, or at least extended over more than one lactation. For example, if a cow's udder was damaged permanently by improper feeding, or by any other environmental cause before she made the first record, that would not alter her breeding value but would lower her producing ability in all lactations. Secondly, some of the dams might, on account of dominance, have permanent abilities higher or lower than correspond to their breeding values. If dominance exists, the daughters will on the average regress farther toward the mean of the herd than would be expected if dominance did not exist, regardless of whether it is the genes for high or for low production which are dominant or whether this is mixed, the gene favoring high production being dominant in some pairs and the gene for low production being dominant in others. Thirdly, some of the genes may in certain combinations produce more (or less) than their average effects; *i.e.*, some genes may have their effects made larger in some genotypes and smaller in others because of complementary, inhibitory, or other epistatic interactions with other genes. When the dam transmits a sample half of her genes to her daughter, many of these special combinations will of course be scattered. To the extent that the constituent genes can produce their effects only when all are together in those peculiar combinations, the daughters generally will not deviate as far from the breed average as would be expected from the permanent abilities of their dams.

Since these three diverse causes for a cow's real ability being higher or lower than her breeding value, together account for only about a third of the variance in the real abilities of a bull's mates (only about one-seventh of the variance in single records), and since it seems certain that some of this one-third is caused by permanent differences in environment, it appears that neither dominance nor epistasis (nicking) were highly important in causing the differences which existed among the cows mated to the same bull. Yet one cannot conclude that either of these two was totally absent unless he assumes that *all* discrepancies between permanent ability and breeding value were caused by environmental effects which differed from cow to cow among the mates of each bull but were the same from lactation to lactation for the same cow.

The Second Study

These data came from the first eight volumes of the Holstein-Friesian Herd Improvement Registry Year Book. All sires having at least six daughter-dam comparisons in which the dam had at least two records were used. There were 209 such sires⁴ with a total of 3010 daughter-dam comparisons. Only the records of fat production were studied. All records were adjusted to maturity and to three-times-milking per day, using the conversion factors developed for that purpose in the Holstein-Friesian Advanced Registry Office.

The data were analyzed by the same procedure used in the first study. Then the process was repeated, this time dividing the dams into a high and a low half on the basis of their second record rather than their first. The first was then averaged with the third and later records, if any, to get an average of the cow's unselected records. This repetition of the analysis, but with the second record as the independent variable, was done merely to see whether the large regression from selected first records to future records was in any way peculiar to or dependent on the fact that the selected record was *the first* record.

The results are shown numerically in table 3 and graphically in figure 1. The regression of real ability on selected record was almost exactly the same (barely under .4) whether the dams were selected on their first record or on their second.⁵

The difference between the daughters is 3.9 pounds larger when the dams were sorted on their second records than when they were sorted on their first. This difference is probably too small and subject to too much sampling error to deserve much attention but it suggests that a cow's second record may be a shade better indicator of her breeding value than her first record is. Among first records 25 per cent of the variance seems to be hereditary in the narrow sense (additively genetic) while the corresponding figure for second records is 30 per cent. These two estimates are independent of each other, since the independent variables (dam's first record in the one case and dam's second record in the other case) do not include any of the same data. The two estimates agree reasonably well. They indicate (as might have been expected) that the moderately low repeatability and other findings from the first study were not peculiar results of using the first record instead of some later record for sorting the cows into "high" and "low" groups. Sorting on the second record gave almost the same

⁴ If any of these were also proved in Iowa Dairy Herd Improvement Associations before 1937 they would have been included in the first study also.

⁵ The close similarity of the two results is not surprising, since many of the cows had only two records. Had this been true of them all, the two regressions must have been equal unless the standard deviation of the first records was different from that of the second records. That is, the two figures are by no means independent estimates of the repeatability in these data.

TABLE 3

Average fat yields in data used for proving sires in Holstein-Friesian Herd Improvement Registry

	Low group	High group	Difference
When divided on mate's first record:			
Mate's first record	403.6	559.4	155.8
Mate's later records	434.4	496.4	62.0
Daughter's records	462.9	482.1	19.2
$\frac{62.0}{155.8} = .40$	$\frac{2 \times 19.2}{155.8} = .25$	$\frac{2 \times 19.2}{62.0} = .62$	
When divided on mate's second record:			
Mate's second record	393.9	549.0	155.1
Mate's other records	438.8	500.3	61.5
Daughter's records	461.6	484.7	23.1
$\frac{61.5}{155.1} = .40$	$\frac{2 \times 23.1}{155.1} = .30$	$\frac{2 \times 23.1}{61.5} = .75$	

results as sorting on the first. Presumably sorting on the third or later records would do the same except as the increasingly stringent selection, entailed by confining the study to cows with three or more records each, might reduce the amount of variance in the real abilities of the population which remained. Permanent but non-transmissible differences between cows are not a large fraction in either case—15 and 10 per cent, respectively, of the variance in single records, or 38 and 25 per cent of the variance in permanent abilities.

Comparisons with Other Studies

In none of the earlier studies in which mates were divided into a high and a low group was the repeatability of single records investigated. Therefore the present studies cannot be compared with them on that point. However, the present figures ranging from just under .40 to .48 do agree well with most studies of intra-herd repeatability in which the data had not extended over a long enough period of time for time trends to be important, or where the cows with early low records had not first been largely culled, as by restricting the study to cows which each had many records.

The earlier studies do permit computing the regression of daughters on dams. The difference between the daughters of the high and the low groups can be doubled and divided by the difference between the records on which the dams were separated into high and low groups. This yields a figure for heritability of differences among those first records, comparable to the 28 per cent for fat and 33 per cent for milk found by Lush and Arnold or to the 25 per cent and 30 per cent found by Norton for fat. Table 4 shows such a summary prepared from the earlier studies known to us. The heritability figures in table 4 are somewhat higher than those we found.

TABLE 4

Summary of evidence on heritability, earlier studies

Author	Characteristic	Difference between high and low groups		Heritability ^a	Notes
		Dams	Daughters		
Gifford	Fat (lbs)	278.7	32.2	.23	21 Holstein-Friesian bulls ^b
Gifford	Fat (lbs.)	240	61.6	.51	18 Guernsey bulls ^c
Copeland	Fat (lbs.)	244	52	.43	20 Jersey bulls ^d
Edwards	Milk (lbs.)	2856	592	.41	23 bulls ^e
Rice	Milk (lbs.)	6373	1815	(.57)	10 bulls, dairy breeds ^f
Rice	Test (%)	1.09	0.47	(.86)	10 bulls, dairy breeds ^f
"Brain Truster"	Milk (lbs.)	5025	945	.38	1 bull with 151 daughters

^a Twice the intra-sire regression of daughters on dams.

^b A.R. records. Each bull had at least 24 daughter-dam comparisons. The mates of each bull were divided into high, medium, and low thirds (approximately). The figures given here are averages computed from Gifford's table 12, giving equal weight to each sire.

^c A.R. records. Each bull had at least 17 daughter-dam comparisons. Mates divided approximately into high, medium, and low thirds. The figures here are averages from Gifford's table 1, giving equal weight to each sire.

^d R. of M. records. Each bull had at least 19 daughter-dam comparisons. Mates were divided approximately into high, medium, and low thirds. The figures quoted are from the summary of Copeland's table 3.

^e Data from British milk recording societies in East Anglia and Lanarkshire and from agricultural college herds at Reading, St. Albans and St. Paul. Mates divided into high and low halves. The figures quoted are averaged from columns 4 and 5 of Edwards' table 3, giving each cow equal weight. As Edwards used average records where available (up to three lactations per cow), the heritability figure shown here pertains to differences between average records rather than single records. If the intra-herd repeatability of single records in Edwards' material was .4, the heritability of differences in single records would be somewhere between the .41 shown here and the .24 which would be approached if every mate had three records.

^f Data are official records from several dairy breeds. Each bull had at least 17 daughter-dam comparisons. For each bull the five "highest producing" mates and the five "lowest producing" mates were selected. Division seems to have been primarily on total fat production and was for milk and test only in so far as they were dependent (statistically) on total fat production. This makes the records for the dams' milk and test come much nearer to representing the dams' real ability than if division into high and low groups had been primarily on the milk records and the test records respectively. The figures for heritability therefore are much too high to be fairly comparable with the others and come nearer to indicating the fraction of the differences in real ability (not records) which are due to additively hereditary differences between the cows.

We do not know whether this difference is statistically significant and needs an explanation. One possible reason for such a difference is that their data may have contained more inter-herd differences than ours. Most of the previous studies were confined to bulls which had an unusually large number of tested daughters. Doubtless that increased the proportion of cases where some of the dams and daughters were kept in one herd while others

were kept in another where the management differed.⁶ This would have contributed an environmental portion to the daughter-dam correlation. Restricting the study to bulls which had very many daughters would also extend the time over which the daughter-dam comparisons had accumulated and would offer a little more opportunity for time trends in management to contribute to the observed correlation. Another conjecture is that our data, being D.H.I.A. or H.I.R. records, may have included a noticeable fraction of lactations made under circumstances abnormal enough that the owner would not have placed the cow on test if the matter had been left to his choice. That is, it is thinkable that other changeable circumstances do play a larger part—and genuinely hereditary differences consequently a smaller part—in such data as ours than in official test figures such as were investigated in most of the earlier studies. In any event the discrepancies between the figures for heritability in table 4 and those in tables 1, 2, and 3, are small.

DISCUSSION

Repeatability

By far the most important source of error in estimating the breeding value of cows from their records is the error which comes from conditions or circumstances which change from lactation to lactation for the same cow and make her production sometimes higher and sometimes lower than it will be if she is tested again under what are intended to be the same circumstances. The herdsman, or other person who knows the circumstances well, might be able to make some kind of allowance or correction for how the record has been affected by the unusual circumstances he knows (*e.g.*, difficult calving, a touch of milk fever or mastitis, freshening in fly time, two weeks of indigestion at what should have been the peak of her production, unusually good luck in avoiding a normal share of mishaps, etc.), but most such corrections will be so subjective that they can hardly be used by an impartial agency such as a breed association or D.H.I.A. supervisor. How far an observant herdsman's knowledge would actually go toward always explaining why a cow produced better in one lactation than she did in another is uncertain.

Lifetime averages are especially effective for correcting automatically the errors caused by unrecorded variations in environment. Variance due to circumstances which change at random from one lactation to another should be only half as large in averages of two unselected lactations, only one-third as large in averages of three unselected lactations, and so on, as

⁶ Our own figures for repeatability and heritability are not absolutely free from that but must be nearly so since most bulls proven in Iowa D.H.I.A. before 1937 and most bulls proven in the first eight volumes of the Holstein-Friesian H.I.R. would have been proven in one herd only.

in single records. The process of averaging does not remove these errors entirely but, if they are random, it sharply reduces them.

Wherever objective correction factors can be developed for important variations in environment, further accuracy can be gained by correcting the original records for unusually good or bad known circumstances, but there are important practical reasons against correcting records too much, lest they get too far from reality.

Wherever simple and impersonal criteria of distinct abnormality can be devised, something might also be gained by omitting records made under circumstances so abnormal that no correction for them can be made (*e.g.*, abortion), but the circumstances justifying such omission would need to be few, definite, and unmistakable.

Heritability

Differences in single records seem to be somewhere between 20 and 50 per cent hereditary, the figures from our own studies being more nearly 25 to 30 per cent. It would of course be desirable to ascertain this figure more precisely and to know whether it is really different in different kinds of records.

Heritability was obtained by doubling the intra-sire regression of daughter on dam. The intra-sire basis was used in order to minimize the environmental contributions to daughter-dam likeness (since it puts most of the data on an intra-herd basis and restricts them to a period rather short for steady time trends to have had much influence) and to avoid analyzing the mating system. Questions of whether the mating system used was materially different from random mating among those selected to be parents were side-stepped by analyzing only the differences between cows mated to the same bull. The extent to which differences between groups of mates were hereditary was thus left unexplored. Genetic differences between groups of mates might be important in a population of partially inbred lines or in a population in which various herds were being selected toward widely divergent ideals.

Our estimates of heritability are a bit too high if there was any general tendency for the owner to give a daughter better environment than the average of the other daughters in his herd merely because he had given her dam better environment than the average of the other dams. Such a primary correlation between the environments of daughter and of dam would have contributed a non-genetic portion of the likeness between records of daughter and dam. We see no way of testing these data to learn whether such intra-herd environmental correlations between daughter and dam did exist but, in view of the feeding and management practices generally followed, we think that such environmental correlations must have

been unimportantly small, save only in the few cases where a sire was proved on daughters from more than one herd.

The definition of "hereditary" used here has included a small part of the epistatic gene effects, in addition to the purely additive ones. Additive gene effects were wholly included (aside from sampling errors), since each daughter gets a half of her dam's genes (and of course whatever average individual effects those genes have in the array of genotypes and environments present in this population), while doubling the regression tends to cancel the halving effects of Mendelism segregation. But only one-fourth of the two-gene epistatic interactions (*i.e.*, the differences between the effects which two non-allelic genes actually do have when together and the sum of their average effects, each considered singly in this population) which are present in the dam would be transmitted to her daughter, only one-eighth of the interactions peculiar to sets of three genes and not exhibited by any one or any two of those genes alone, only one-sixteenth of the four-gene interactions, etc. Hence the method of analysis used here has included in the 25 to 30 per cent of the variance which was "hereditary" not only the truly additive effects of genes but also about one-half of the effects which depended on the interactions of two genes, one-fourth of the effects of three-gene interactions, one-eighth of the four-gene interactions, etc. That such interactions may exist cannot be denied but in these data they can hardly have been very important, since one-half of the two-gene interactions, three-fourths of the three-gene interactions, seven-eighths of the four-gene interactions, etc., are included with the dominance deviations and the permanent environmental effects, which all together constituted only about 10 to 15 per cent of the variance.

Changing the Population Average by Culling Cows

Something about the rate at which the average production of a breed can be increased by culling low-producing females can be estimated from the fact that the intra-sire regression of daughter's record on dam's selected single record is something of the order of one-eighth to one-sixth in our data—a little higher in some of the other studies cited. The annual turnover in dairy herds is around 25 to 30 per cent of the average number of cows in the herd during the year. At least a third of those removals and possibly more than two-thirds are involuntary, being due to such things as old age, deaths, sterility, sales which would not occur if the cow were known to be well along toward calving, etc. If the voluntary selection which can actually be practiced is equivalent to discarding each year one-eighth of the cows which have the lowest records, the heifer calves sired the next year by the same bulls would average about two to four more pounds of fat per year when they come into production than the heifer calves from the preceding year would average. Seath in a study (10) of Iowa and Kansas D.H.I.A.

herds found that culling was not quite as intense as is assumed here. While a closer approximation is much to be desired, we think that these data and general considerations justify the opinion that the maximum amount of such culling of low producing cows as would be possible in herds generally, would not be enough to raise the average genetic productiveness of a whole breed at a rate as fast as three more pounds with each additional year. How rapidly the composition of a whole dairy population could be changed by the selection of bulls is another story and a much more complicated one.

Validity of Sire Indexes

Estimating the breeding value of a bull as equal to the average of his daughters plus the increase of these daughters over their dams rests on the genetic principle that the genotypes of the offspring tend to be midway between the genotypes (breeding values) of their parents and, in practice, on the inference that the average of the daughters' records equals the average of their genotypes and that the average of the dams' records equals the average of their genotypes under the general environmental conditions which are thought to have prevailed in that herd.⁷

⁷ Because the interrelations between test (f), total milk (M), and total fat (F), are multiplicative but the segregation and recombination processes of heredity are additive, sire indexes are not quite identical when computed directly for F and when computed indirectly by multiplying together the indexes obtained separately for f and for M . The variables M , f , and F constitute a closed system in that when the values of any two of them are specified the values of the third is automatically fixed, but this does not of itself show which (if any) of them can be considered as primary or causal to the other or others. Several investigators have maintained that M and f are inherited independently and that F results from their interactions. This would make the indirect computation of indexes for F more nearly correct. Other investigators, notably Gaines, have maintained that the faint negative correlation (not far from $-.2$) generally observed between M and f is large enough to require explanation and is just about the size which would be expected if total energy yield (which is so closely correlated with F as to be almost synonymous, at least on an intra-breed basis) were primary. This would make the direct computation of indexes for F more nearly correct.

Whatever the physiological truth about that may be, the consequence of using whichever is the less accurate method of computing the sire index will be to throw into the epistatic portion some of the variance which would have been truly additive if the more accurate method had been used. The indirectly computed index for F exceeds the directly computed one by $2xy$ where x is the daughters' average test minus their dams' average test and y is the daughters' average milk yield minus their dams' average milk yield. Thus the indirectly computed index is larger than the directly computed one when x and y are both positive or both negative, but falls below it when x and y are of opposite sign. The actual size of $2xy$ in D.H.I.A. data was studied in a sample consisting of the first sire on each page in the list of proved sires published in August, 1940, as Miscellaneous Publication 393 of the U.S.D.A. For this group of 205 sires the average value of $2xy$ was -1.6 pounds and its standard deviation was 5.3 pounds. The two most extreme values found were $+18$ and -27 but only nine per cent differed from zero by as much as ten pounds and three-fourths of them were less than five pounds away from zero.

In the practical use of the index two sources of error are encountered. The first is the sampling nature of inheritance whereby the genotype of any one offspring may be better or poorer than the average genotype of its parents. Since the mechanism of inheritance makes such sampling errors always random, they are as likely to be plus as minus in each case and this source of error can be made unimportantly small (especially for characteristics affected by many genes) simply by increasing the number of offspring and seeing to it that they are an unselected sample.

The second difficulty is that the genotypes of the offspring and of the dams are unknown and can be estimated only from their records (phenotypes). So far as the differences between the individual's genotype and the individual's record are random, errors from this source also are as likely to be plus as minus in each case and can be made unimportantly small by increasing the number of dams and offspring. But in actual practice many of these discrepancies between genotype and record are not random but are more or less consistently biased in one direction. When these biases are large, only a little accuracy is gained by increasing the number of daughter-dam comparisons beyond three or four. Examples of such biased errors are: (1) that the daughters or mates of one bull are kept under an environment more favorable and another bull's daughters or mates are kept under a less favorable environment than the man interpreting the data thinks; (2) that the daughters or the mates used to prove the bull are a selected group—in which case their records tend to be better than their real abilities; (3) that sometimes only the highest record of each cow is used to represent her—in which case the records are generally better than what the cows will really do in the future (a source of bias which is more extreme for the mates than for the daughters since the former will generally have more records from among which to select a high one); and (4) certain rare (and therefore generally unimportant) genetic situations, such as an extreme change from intense inbreeding to wide outbreeding or the reverse, in which cases the daughters may be expected to show more heterosis or more inbreeding depression than their dams.

If the daughters were unselected their average phenotype can be taken as equal to their average genotype under the general conditions of that herd. The possibilities for error in doing this are: (1) random plus or minus errors caused by individual fluctuations in the environment which

While of course it would be intellectually satisfying and scientifically interesting to know more certainly the relative accuracy of the two methods, yet for the practical purposes of choosing or rejecting one sire as compared with another the differences between the two methods are unimportantly small compared to sampling errors and to the ever-present possibility of incorrectly appraising differences in the general environment which prevailed either for the daughters or for the mates of any of the bulls being compared. The difference between these two ways of computing an index for F is of interest chiefly because it has sometimes been interpreted as challenging the general validity of indexes.

affected the various daughters and which may have made the actual records of some higher and of others lower than would generally be typical of cows with such genotypes, and (2) the general environmental conditions of that herd may have been better or poorer than is realized or than in the herd in which was proved another bull being compared with this one. Errors from the first source can be made unimportantly small merely by increasing the number of daughters. There is no such automatic way to guard against errors from the second source. One can only estimate this from records and observations of the feeding and management prevailing in the herds concerned. Such estimates can hardly be perfectly accurate but they are usually helpful, sometimes very much so.

It is now pretty well understood that for the progeny test to be unbiased the daughters should be an unselected sample—although that was not so generally conceded 20 or 30 years ago when arguments were frequently advanced that even a single high-producing daughter proved that a bull *did have the ability* to transmit that level and therefore he should be judged more by his best daughter than by the average of all—with substantially the same logic as is still sometimes offered in favor of using a cow's best record instead of the average of all her records! Yet it is never known that an actual group of daughters was absolutely unselected. The nearest approach to that is when every daughter born is tested. Even then some bulls might have transmitted more zygotic lethals, resulting in resorptions, abortions, or stillbirths, than other bulls. Often some of the daughters born alive die or are barren or are sold before reaching breeding age and no production record from them can ever be had. Probably most of these omissions result from accidents or circumstances not related to the heifer's innate producing ability and hence do not tend to bias the average of the sire's progeny, but at least a few of these omissions result from constitutional weaknesses or culling for suspected low production, in part hereditary and having some bearing on the sire's breeding value. In short, natural selection *must* and intentional selection *may* impinge on the group of daughters to such an extent that the statement that the daughters must be unselected expresses an ideal which can be approached more or less closely and is very much worth striving for, but is rarely known to have been attained absolutely.

The mates have been exposed to all this selection and more, too. The more times a cow calves, the more chances she has to appear as a dam in the proving of a sire. For example, if about one-third of all calvings result in heifer calves which are raised and tested in the proving of some sire (probably a rather liberal estimate in view of stillbirths, death losses and barrenness among the one-half—approximately—of the calves which are heifers), only one-third of the cows which are culled before their second calving will appear as dams in daughter-dam comparisons. Of those cows

which calve twice, one-ninth would appear twice and four-ninths once as dams in daughter-dam comparisons, while four-ninths would not appear at all. Of cows which calve three times, one twenty-seventh would appear three times, six twenty-sevenths would appear twice, twelve twenty-sevenths would appear once, and only eight twenty-sevenths would not appear at all in sire proof. Thus the dams in the usual sire proof contain a disproportionately large share of cows which for many lactations escaped death or culling, and a correspondingly small share of those which left the herd early.

If the dams were selected solely on their past records in that herd, then the best estimate of each dam's genotype (G) is:

$$G = A + \frac{nh(D - A)}{1 - r + nr}$$

in which:

A = the average of herd in which the dam was tested or, more precisely, the average of the whole group out of which the dams were selected.

n = the number of lactation records for that cow.

D = the average of those n records.

h = the heritability of intra-herd differences on the basis of single lactations (*e.g.*, .28 for fat in these Iowa D.H.I.A. data).

r = the intra-herd repeatability of single lactations (*e.g.*, .43 for fat in these Iowa D.H.I.A. data).

For strictest accuracy the average of the above values for G should be used rather than the dams' actual average in computing the sire index, the formula for which then becomes:

$$\text{Index} = \text{Twice the daughter average} - A - \text{Average} \frac{nh(D - A)}{1 - r + nr}$$

Now if the dams were unselected the last term tends toward zero, the plus items and the minus items in that average being about equally numerous and tending to cancel each other and it doesn't matter whether D or A is used, as they are nearly equal. But if the dams were themselves a selected group D will exceed A more often than not and the last term will tend not toward zero but toward some figure determined primarily by the size of $D - A$ and of h but also affected by n and by r . The sum of the last two

terms lies between A and \overline{D} , being $\frac{nh}{1 - r + nr}$ of the way from A toward \overline{D}

when n is the same for all dams. Where lifetime averages for the dams are used, n will generally average between 2 and 4. The values obtained for

h and r in the present study give values for $\frac{nh}{1 - r + nr}$ ranging from .36 to .45 when n is 2 and from .49 to .55 when n is 4. It therefore appears that in general the correct amount to be subtracted from twice the daughter average is not far from half way between A and \overline{D} (perhaps a bit nearer the

former), if the values we found for h are typical. Lower values for h would move the correct figure nearer to A , while higher values would make it nearer to \bar{D} .

Whether it would be worth while to correct each cow's records to obtain her most probable breeding value (G) to use in place of D in the sire index would depend upon how much more accurate the index is thereby made and upon the labor (mostly clerical, of course) and other costs of making the correction. Where $\bar{D} - A$ is small (*i.e.*, where the dams were not in fact a highly selected group) there has been, of course, little error introduced by selection of the dams and little is to be gained by any correction for it. Where $\bar{D} - A$ is large the use of \bar{D} in the index, without making any allowance for the regression of dams' probable breeding values toward the herd average, makes the sire's index lower than if he had been tested on unselected dams.⁸ This will tend to make a sire's index lower than is fairly comparable with a cow's record but, for comparing one sire with another the existence of $\bar{D} - A$ introduces error only to the extent that $\bar{D} - A$ varies from sire to sire. How much variation is there in the intensity with which the mates of different bulls actually were selected? Seath's study (10) of 147 Iowa and 37 Kansas D.H.I.A. herds showed that those cows which remained in the herds at least one more complete year had averaged in the preceding year 16 and 14 pounds, respectively, more fat than the entire herd. (This would be $\bar{D} - A$ of the preceding formulae, so far as any one year's selection was concerned.) There were statistically significant deviations in culling intensity from herd to herd and yet these deviations were not extreme. For example among the 37 Kansas herds this figure varied only from zero to 33 pounds. We do not think such variations will often be extreme. In actual practice no one intentionally selects for low production. Variations in the intensity with which the mates of a bull have been selected occur only because men vary in the importance which they attach to production records, or in their biological and financial freedom to select. Only on rare occasions, as when someone with sufficient wealth assembles a foundation herd by picking a few high record cows from each of many herds, or at the opposite extreme when a man has permitted buyers to top out his herd and starts with the low record remainder to build his herd again, would variations in intensity of selection of mates be extreme.

Where it is suspected that one sire has been used on mates selected with unusual intensity, that can be verified by comparing the records of those mates with the average of their herd in the same years. That will entail more comparison of each cow's record with the herd average than is yet

⁸ The increase of daughters over dams is even more severely biased by this circumstance. This seems to warrant some optimism in interpreting such findings as that nearly thirty thousand daughter-dam comparisons in the germ plasm survey reported in the 1936 Yearbook of the Department of Agriculture showed an average decrease of one pound of fat from the dams' records to the daughters' records.

customary, but perhaps no more than should be done. If such a comparison shows that correction for differences in intensity of selection is needed, the mates' records in each case can be brought nearer to their herd average by the formula for estimating G from D . There would be some complications in determining *exactly* how to compute the age-corrected herd average (A) for the comparable years but these will not prevent an approximation accurate enough for the present purpose. Perhaps we will eventually come to consider all records more on that basis, as has been urged to a limited extent in Scotland (byre average) or in Germany (Stalldurchschnitt). In small herds A will be erratic because of sampling errors. The values of h and r should be known with greater precision and, if they really vary much, their values in different kinds of populations need to be better known. In some cases selection may have been primarily for things other than the dam's records and, if those things were correlated with production, this has the effect of making the fraction $\frac{nh}{1-r+nr}$ nearer to unity (which would completely justify the use of \bar{D} and the omission of A from the index) than it is when selection is entirely on the basis of records.⁹

Because increases in n make the fraction $\frac{nh}{1-r+nr}$ nearer to unity and diminish the size of $D-A$ which can be attained, the use of lifetime averages tends automatically to diminish (although it does not entirely eliminate) the bias which selection of dams introduces into the index computed as if \bar{D} were identical with G .

Most of the discrepancy which selection of dams can introduce into the sire index disappears if the dams' records are properly corrected for incomplete *repeatability* (i.e., even without going so far as to correct for incomplete heritability of permanent differences). For example in the present Holstein H.I.R. data, if a composite index for the high dams and one for the low dams is computed, using for each dam her first record (the one on which she was classified as high or low), we get with the low record dams an index of 522.2 and with the high record dams an index of 404.8, the difference being 117.4 which seems alarmingly large, since the bulls are the same. But if we discard the selected record and use only the future records of the very same cows (thus correcting completely for imperfect repeatability) the index with the low dams is 491.4 and with the high dams is 467.8, the difference being only 23.6 pounds which is a bias, to be sure, but is small compared to sampling errors and other sources of error in actual

⁹ It should not be inferred that this hints at a short cut to more rapid improvement in production by selecting for something other than records of actual production. What is gained by increasing the size of $\frac{nh}{1-r+nr}$ will be more than lost by the smaller size of $\bar{D}-A$ which can be achieved if the dams are selected for something correlated with productivity rather than directly for their own records of production.

practice. Moreover, even this small difference was produced only by differences in selection of mates more intense (high half versus low half) than would often if ever be met in practice. That this remaining difference (23.6) does not approach zero is due to the fact that some of the differences between the records on which the selection was based were permanent for the cows concerned but were not transmissible to their daughters—i.e., were due to permanent effects of environment, to dominance or to epistatic gene interactions. When these cows are sorted on the second record, the difference between the indexes on the “high” and the “low” groups is 108.9 when the mates are represented by the record on which they were sorted, but only 15.3 when the same cows are represented by all their other records, thus fully discounting the differences between record and real ability. In the Iowa D.H.I.A. data the differences in the fat index were 73.9 when the mates were represented by their selected records but only 15.4 when the same cows were represented by their other records. The difference in the milk index was 1765 pounds with the selected records but only 410 pounds when the very same cows were represented by their other records. These small remaining differences will disappear, too, if heritability (h) is known well enough that correction for it can be made.

It is pertinent to inquire whether there would be less error if A were used in place of \bar{D} in the ordinary form of the index. This would be a little like the Swiss practice of proving a sire by comparing his daughters with the average of the association in which they were tested rather than with their own dams. This is done there to avoid penalizing a bull whose daughters are grazed at the higher altitudes and to avoid giving an unearned premium to the bull whose daughters are kept mostly on the richer pastures in the lower valleys. There will be less error in using A alone in place of \bar{D} alone whenever the average value of $\frac{nh}{1-r+nr}$ is less than one-half. With n ranging from 2 to 4 and with the magnitudes of h and r which are usually encountered this fraction will generally be not far from one-half, probably a bit lower more often than it is higher. In general the larger n and the more h exceeds half of r , the more the advantage swings from using A toward using \bar{D} if either must be used alone. Incidentally one can partially justify extending the index to include daughters out of untested dams, using the herd average (A) in place of the record of each such untested dam and D for each tested dam. This would remove one moderately important practical limitation on the use of indexes. Naturally it would be a coincidence if this happened to give \bar{D} and A *exactly* the proper weights but the composite figure used would be somewhere between them, as the theoretically correct figure would be. Also of course in practice it would be necessary to be sure that the untested dams were in fact untested and that the records were not merely omitted because that would thereby give the bull a higher

index! Perhaps one wouldn't often know A where the dams were untested?

The most effective procedure for giving a bull a falsely high index through selection of his mates would be as follows: Assemble a group of cows which have only one record and that record an unusually low one in the herd in which it was made. Never test these cows again, or at least never use their subsequent records. Breed the bull to them and test his daughters when (some three or four years after the plan is started) they begin to freshen. Merely to state these requirements is enough to show that it would never be profitable for an unscrupulous man deliberately to undertake this with the hope of making a profit on the extra price he would then get for the high index of his "proved" sire (if still alive) some four or five years after the plan was begun! Especially would this not appear attractive since the bull's daughters would probably not themselves be especially profitable since they would be out of dams poorer than average, even though not as poor as their records. Moreover the requirements (becoming more widely adopted) that lifetime averages must be used in proving sires, and that all cows in the herd must be tested, go far toward minimizing even this possibility.

*The Sire Index Compared with the Daughter Average or the
Daughter-Dam Difference*

Space prevents a detailed comparison of the various ways proposed for comparing proven sires with each other, but the source of error which is the main object of this study,—more intense selection of the mates of some sires than of others,—biases the daughter-dam difference most, among the commonly used measures of a sire's worth. Selection of the dams makes the increase of daughter over dams too low by the quantity $(\bar{D} - A)\left(1 - \frac{h}{2}\right)$ where the dams have only one record each and no correction for imperfect repeatability or heritability is made. The daughter average is biased in the opposite direction by the quantity $(\bar{D} - A)\frac{h}{2}$ which favors the bull mated to the most highly selected cows. Since the index is simply the sum of the daughter average and the increase of daughters over dams, these two opposite biases partly cancel each other in the index, leaving the net bias equal to $(\bar{D} - A)(1 - h)$. For values of h lower than two-thirds this bias will be larger, when expressed in pounds, for the index than for the daughter average. But the standard deviation of indexes will be larger than the standard deviation of daughter averages,¹⁰ ranging from only a

¹⁰ The ratio of the standard deviation of the index to the standard deviation of the daughter average in populations in which each daughter and each dam are represented by one unselected record and the standard deviation of single records of daughters is equal to the standard deviation of single records of dams is:

little larger, in populations where the herd differences are extreme and the number of daughter-dam pairs is very large, to about twice as large in populations where averages differ little from herd to herd. When the bias from differences in the selection of dams is expressed relative to the standard deviation of whatever measure is used for the sire (as it should be for comparing the practical importance of a source of error), this bias would be equally serious in the daughter average and in the index when h is somewhere around .4 to .6. When the dams have more than one record each, the h of these computations would be replaced by $\frac{nh}{1-r+nr}$ which is larger.

Therefore the general situation on this point is that the daughter average is usually a little less affected by selection of dams than the index is, especially when h and n are small, but this may be reversed when h is large relative to r , and when the dams have several records each.

The daughter average is most vulnerable and the daughter-dam difference is least vulnerable to errors from wrongly appraising differences in management and general environment from herd to herd, with the index again being intermediate but somewhat nearer to the daughter average.

Adjusting a Cow's Record for Imperfect Repeatability or Heritability

Corrections for imperfect repeatability are not usually made in practice, except when comparing individual cows which do not have the same number of records. For most other purposes one will be using an average of the records of several cows. Correcting for imperfect repeatability would lower some of these toward the herd average but would raise others. If the group of cows was unselected, these plus and minus corrections would tend to

$$\sqrt{4 + \frac{1 + (n-1)(v-4u) - 4t}{1 + (n-1)w}}$$

in which n = number of daughter-dam pairs.

w = correlation between paternal sisters.

u = average correlation between daughter and a mate of her sire, other than her own dam.

v = correlation between cows mated to the same sire.

t = correlation between daughter and her own dam.

All these correlations are computed as if the whole population in which these sires were proven was a single unit (*i.e.*, they are not intra-herd correlations). u and v will be approximately the same size and will be roughly equal to the average correlation between herd mates. They are measures of the amount of herd heterogeneity in the population and in most dairy populations thus far studied are something of the order of +.2 to +.4.

w will be larger than v by about $\frac{h}{4}$. t will be larger than v by about $\frac{h}{2}$. Where each cow is represented by several records all four of these correlations would be larger and this ratio would be noticeably smaller when n is very small but would not be changed much when n is large.

cancel each other and, therefore, little would be gained by making them. But where the cows were selected partly because they already had records higher than others in the population from which they were taken, more downward than upward corrections for imperfect repeatability should be made and therefore these will not tend wholly to cancel each other. The formula for making the repeatability correction is that a cow's most probable future ability is $\frac{nr}{1-r+nr}$ times as far from the average of the population as the average of her n completed records is, with r being the repeatability coefficient or correlation between different single records of the same cow in that population. Where $r = .4$, which is close to the value generally found for intra-herd repeatability, this fraction reduces to $\frac{2n}{2n+3}$, which is an approximation close enough and simple enough for general use.

If it is desired to estimate a cow's breeding value instead of her own ability (that is, to discount not only the temporary environmental differences in records but also differences which are permanent in that cow but not transmissible), the r in the numerator must be replaced by the heritability fraction (h) which, of course, will be somewhat less. Thus in the Iowa D.H.I.A. data the fraction for estimating the real future ability of the cow to produce fat becomes that her real ability probably is $\frac{.43n}{.57 + .43n}$ as far from the herd average as the average of her past records is, while her breeding value should be estimated at $\frac{.28n}{.57 + .43n}$ as far above or below the average of her contemporaries as her actual records average.

Age Corrections

Incidentally, these data have some bearing on the approximate correctness of the age conversion factors used. In the Iowa D.H.I.A. data the dam's first production record (not always her actual first lactation) averaged 389.4 while the later records averaged 386.0. This slight decrease could be interpreted as an average bias in the age correction factors used but it could also be interpreted (and more logically it seems to us) as the result of selection and subsequent regression toward the herd average. Cows with only one lactation could not appear in these data. If some cows were culled because their first lactation was low and before they could complete a second lactation, that would have made the average of first lactations here a bit higher than the abilities of the cows which made them. Cows culled because of unusually low first records could not be there to show the expected regression upward, while cows with unusually high first records would be kept and would show the corresponding regression downward. If the age corrections were completely unbiased and repeatability of single

records were .4, this decline of 3.4 pounds is the amount which would be expected to result from selection equivalent to discarding before they could complete a second lactation the five per cent of the cows which had completed the very poorest first records. This is just a little less intense than the selection Seath found. The average of later records (386.0) is not biased by selection, since all cows with two or more records could be included, provided only that they had tested daughters. In the Iowa D.H.I.A. data the age-corrected first milk records average 9736 and the later ones 9746. If selection for milk records was practiced, some compensating circumstance conceals the results of it, except that the daughters average only 9574. That might be considered either as evidence of selection among the dams or as indicating that the sires did not average as high in breeding value for milk production as their mates.

In the Holstein-Friesian H.I.R. data the first records average 481.5 and the second records average 471.4. This difference is equal to the amount which would be expected if the age correction factors were correct but in these H.I.R. herds selection between completion of the first lactation and completion of the second lactation had been of an intensity equal to culling seven or eight per cent of the cows with the very lowest first records.

Ward and Campbell in an interesting recent paper (11) interpret their data as showing that age corrections should be made by a regression equation and not by multiplication with a percentage correction factor. However, their method really corrects *both* for *age* and for *incomplete repeatability*, in a single operation. The equation for predicting from her first record (X) what a cow will most probably produce (Y) under a fresh sample of environment when mature is simply:

$$Y = (1 - r)A + raX$$

where a is the percentage age-correction factor appropriate for the age when record X was made, A is the mature average of that population, and r is the repeatability of single records. This corresponds to the equation under Ward and Campbell's table III except that their r of .64 is the correlation between first record and the average of the four later ones. This .64 corresponds to a repeatability of not far from .51 between single records,—.4139

is approximately equal to $\frac{4r^2}{1+3r}$, a little more or a little less according to inequalities among the various r 's and c 's. This .51 is for the population as a whole and therefore (to the extent that these New Zealand herd averages differed from each other either because of environment or genetically) is larger than the intra-herd r 's which we have been discussing here. The findings of Ward and Campbell do not conflict with the idea that corrections for age alone should be made by multiplying the actual record by a factor appropriate to that age.

Variability of Records, Abilities and Breeding Values

A cow's record, being the result both of her own ability and of the impinging environment, is more variable than either of these constituents. Similarly variation in real ability is greater than variation in breeding value. Quantitatively this is shown in Figure 2, using for illustration the

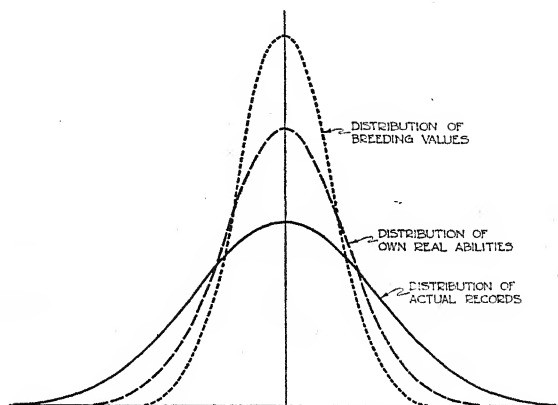


FIG. 2. Normal curves of equal area but with standard deviations in proportion to 100, 66, and 53. While it is not likely that the distributions of records, real abilities, and breeding values are exactly normal (distributions of biological data often being skewed a bit to the right or left for genetic or physiological reasons), yet they are nearly enough normal for this diagram to illustrate with fair accuracy the general principle that breeding values differ less than individual abilities and these in turn (at least for milk and fat production) differ far less than records of actual production. In general the cows with the very best records aren't as good as their records and the cows with the very worst records aren't as poor as their records.

Iowa D.H.I.A. data on fat where the repeatability was .43 and the heritability was .28. The standard deviation of real abilities is $\sqrt{.43} = .66$ per cent as large as the standard deviation of single records. The standard deviation of breeding values is $\sqrt{.28} = .53$ per cent as large as the standard deviation of single records. This leads to the estimate that the intra-herd variation in breeding ability (σ_G) was about 34 pounds of fat in Iowa D.H.I.A. cows and 49 to 53 pounds in H.I.R. data. About half of the difference between these two standard deviations would be expected to result from the H.I.R. data being corrected to a three-times-per-day milking basis. The rest of the difference may not be significant but it suggests that average H.I.R. management conditions are such as to expand the variation in records to a wider range than under C.T.A. conditions without, however, altering noticeably the proportion of that variation which is due to differences between the cows.

In any event these estimates give grounds for careful scrutiny of cases where a bull is supposed to have raised the production of his daughters over

their dams by more than 50 pounds of fat. Even in the H.I.R. conditions only one bull in 40 would be expected to do as well as that if his mates were average cows of the breed and not one in millions could achieve a real genetic increase of as much as 100 pounds. This is not to cast doubt on the accuracy of the records when such cases are reported, but to call attention to the great probability that the daughters were managed better than their dams, or were a selected sample, or that these dams had records far below their contemporary herd average, or that the number of daughter-dam pairs was not large enough to keep chance from playing a large rôle. The loopholes in sire "proof" are often large.

SUMMARY

The major source of error in estimating the breeding worth of cows or in interpreting the progeny test of bulls from production records is in environmental circumstances known and unknown which may make one record higher or lower than another, even for the same cow kept for another lactation under what are intended to be the same conditions.

Dominance, epistasis, and individual peculiarities of environment which affect permanently a cow's ability to produce fat are of minor importance in causing differences in the records used to prove sires. All three of these sources of variation combined are only about half as important as permanent differences which are simply transmissible from dam to daughter.

When the mates of a bull were divided into a high half and low half on the basis of one record and then the later records of the mates and the records of their daughters were compared, the sources of variance in single records were found to be as follows:

Percentage caused by	Iowa D.H.I.A. data (first lactation)		H.I.R. data Holstein-Friesian	
	Fat	Milk	Fat in first lactation	Fat in second lactation
Temporary variations in environmental conditions	57	52	60	60
Permanent but non-transmissible differ- ences between cows	15	15	15	10
Hereditary differences between the cows	28	33	25	30

Differences in the intensity with which the mates of various sires were selected will bias sire proof, especially the daughter-dam difference and to a lesser extent the index and the daughter average, but this bias is rarely large enough to need much correction in actual practice. Nearly all of that bias can be removed, where necessary, by correcting the mates' records toward the average of the group from which they were selected, so as to

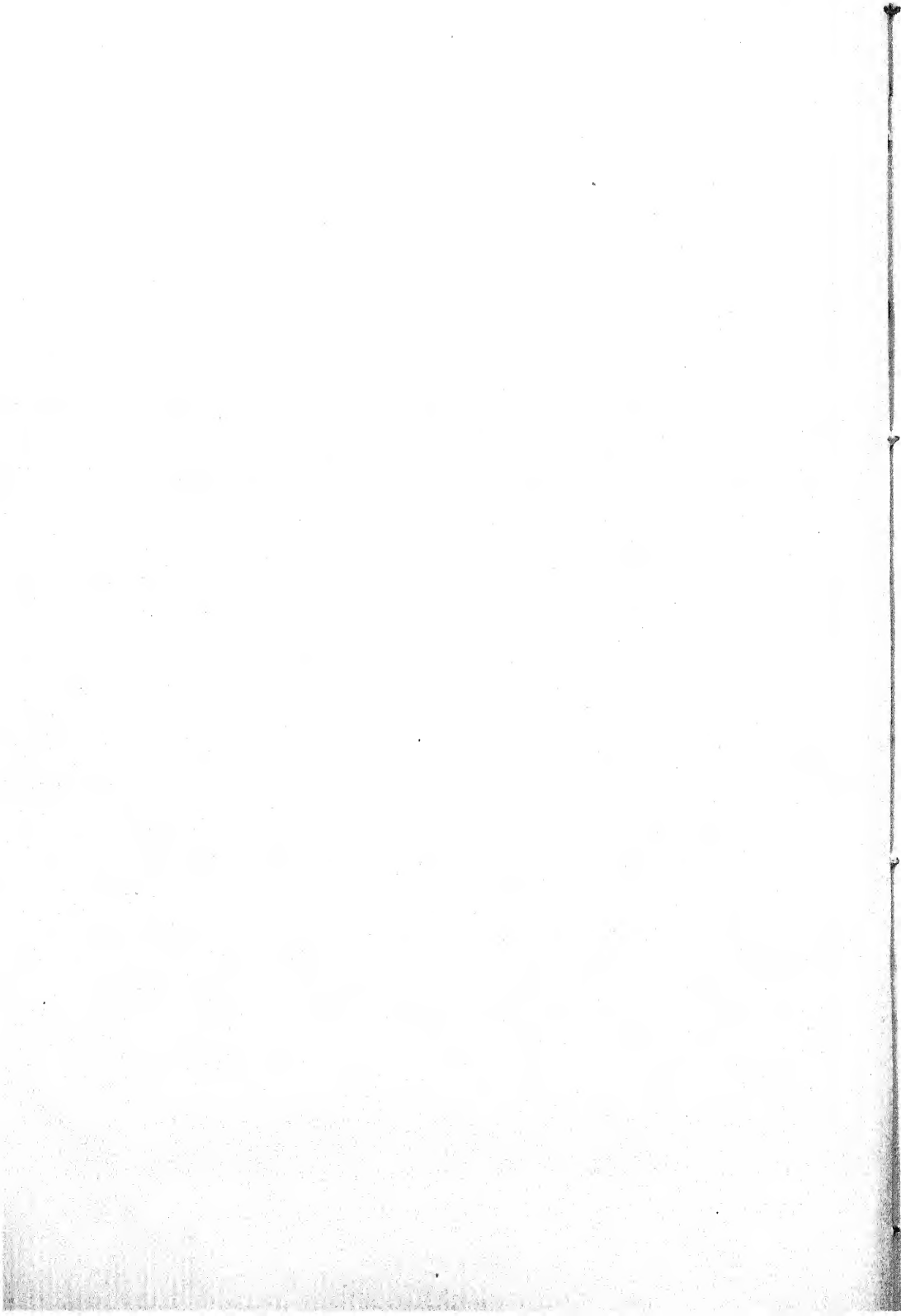
allow for the regression of real ability or transmitting ability on selected record.

The use of lifetime averages automatically corrects for much of the bias which in selected groups exists between the records on which they were selected and their real abilities.

Daughters whose dams were untested can be included in sire indexes, by using the herd average in place of the record of each such dam. Such procedure is more likely to improve than to lower the accuracy of the index, although there is some risk of the latter.

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DAIRY PRODUCTION MILESTONES

W. E. KRAUSS

Ohio Agricultural Experiment Station, Wooster, Ohio

On June 17, 1938, Dr. H. B. Ellenberger of the University of Vermont presented a paper in the grove next to the dairy building at the Ohio Experiment Station on "The Contribution of Production Research to the Advancement of the Dairy Industry." It seems rather ironical that three years later I should be asked by the General Program Committee to give a paper on the campus of the University of Vermont on "Dairy Production Milestones." Whether or not the Program Committee was justified in this move will be for you to decide later. Whatever your decision may be it remains fitting that we meet in a state where 60 per cent of the agricultural income is derived from milk, and on a campus from which considerable of our dairy knowledge has emanated.

To attempt to go back to the beginning of time and trace step by step the advances that have been made in the dairy production field would be not only too great a task but superfluous. For the purpose of this paper it is not necessary to know that the oldest records of dairying go back to 6000 B.C.; that the Old Testament has many references to cattle, milk, butter and cheese; that all cattle belong to the genus *Bos* (from which the affectionate term "Bossie" may have originated); nor even that trench silos existed in the days of Caesar. These are matters for classroom discussion—things with which you who teach are more familiar than I. We are more concerned with the dairy production picture as it exists today in this country, the events that have made it what it is, and what the future may hold.

In the beginning, and for some time later, the producer was the whole show in dairying, even after man learned how to make butter and cheese. Until about the middle of the nineteenth century practically all butter and cheese was made on the farm. The first cheese factory was established in 1851 and the first creamery came into existence five years later. From then on the shift was rapid and we find in the official records fifty years later that creameries were making a billion and a quarter pounds of butter a year and that cheese factories were turning out 300,000,000 pounds of cheese annually. What a change had come about!

This shift in labor distribution and responsibility was of great significance to the producer on the farm, for it allowed him more time for improving his cattle and his land. This, plus the coincidental developments occurring during the same period, brought about more changes in the course of

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one century than had occurred in the entire period of history preceding. Among the more important developments assisting in this change were inventions such as the cream separator, the mechanical milker, and refrigeration; discovery of the principles of genetics; the establishment and development of breed associations and other associations designed to spot superior animals and eliminate the boarders; development of feeding standards, nutrition, bacteriology, and chemistry, with the Babcock test the most significant chemical procedure affecting the conduct of the entire industry. We cannot forget either that improvements in agronomic practice contributed much to this rapidly changing dairy world.

The establishment of Federal and State-supported agricultural institutions and a Federal Bureau of Dairying has, of course, made it possible, through the agencies of research, teaching and extension, to discover the facts around which any sound enterprise must be built and to disseminate these, along with other bits of information, to all who would like to know, thus supplementing the original tool of progress—experience.

As a result of the milestones already mentioned and the many more yet to be indicated, where are we today? This can best be answered by comparing the farm dairy enterprise today with what it was 100 years ago, just before the great transition began. There wasn't much to the dairy business 100 years ago. Each farmstead had a few cows that were housed in the winter, when they were usually dry, milked when convenient, and fed whatever happened to be available—grass during the summer, and a little grain, straw, roots, and potatoes during the winter. Later, when corn was common, many cows wintered on corn fodder and a straw stack. The milk was consumed as such by the family or as butter and cheese, except in the case of large herds in the native pasture regions. A good cow in those days was expected to produce about 160 pounds of fat in a year. The highest record known was 12,000 pounds of milk a year. The average yearly production was about 2,000 pounds. Breeding and feeding were haphazard. There was no farm machinery. There were no tower silos. There was no alfalfa; there were no soybeans; there were no commercial fertilizers; there were no commercial feeds; there were no proven sires; there were no county agents, dairy extension men, dairy professors, or dairy specialists. The dairymen were on their own and cattle declined instead of improving until a few wise men like Wintrop Chennery, Phillip Dauncey, and W. G. Duncan imported cattle from abroad, kept them pure, and bred for desirable characteristics.

Today it is different and much more complicated. The dairyman of today must be a smart man to succeed, for he must be an expert in at least four major activities: farm management, production, marketing, and mechanics other than the baling wire type. Given some available labor, capital, land, buildings and fences, machinery, cows, and a few minor sources of cash income such as hogs, chickens, cash crops, or outside work,

the dairy farmer must utilize these resources in such a manner as to realize a profit.

The problems of production are concerned not only with milk but with calves and with crops. For all three of these disease control must be understood. Marketing machinery must be understood in order to make the best disposition of the product. Systems of buying milk must be considered, although we could hardly expect all the dairy farmers of the East North Central States, of which Ohio is one, to know that the "code" price of 100 pounds of milk delivered to evaporating plants is obtained by successively multiplying the monthly average wholesale price of 92 score butter at Chicago by 6, adding 2.4 times the monthly price of "Twins" on the Wisconsin Cheese Exchange, dividing by 7, adding 30 per cent, and multiplying by the butterfat content of the milk. The buying habits of the consumer must be known and sanitary requirements must be observed. For all this many skills are required, not the least of which is the ability to operate and repair farm machinery, without at the same time becoming a machine slave like the farmer, who, when the power failed while running a milking machine, called out the barn door, "Does anyone around here know how to milk a cow?"

Just how involved the dairy production field is can best be illustrated by the curriculum we expect a student majoring in dairy production to follow. At our meeting this year a report will be made on the subject matter that should be covered by a dairy production major. In the field of veterinary medicine alone it will be recommended that he attain quite comprehensive knowledge regarding anatomy, physiology, hygiene and preventive medicine, and pathology. Theoretically he should become fully versed in veterinary medicine as it applies to dairy animals. Multiply this one activity by the many others comprising the dairy production field and you begin to realize how complicated our course of training must be. The importance of physiology in understanding the chemistry and physics of milk secretion, with its intricate hormone relationships, is only too apparent in the literature of recent years, and advancement in both of these fields of endeavor constitutes a momentous milestone.

In spite of the complexities of the business, much progress has been made. In 1940 about twenty-four million cows in the United States averaged 4,575 pounds of milk and 181 pounds of fat. This is almost two and a half times the average of a hundred years ago. But that isn't the whole story, for in 1939 cows in dairy herd improvement associations averaged 7,977 pounds of milk and 323 pounds of fat. Also, we can now boast of a world's record production of 38,606.6 pounds of milk and 1,402 pounds of fat, made by Carnation Ormsby Butter King. This kind of boasting may not be of the right kind, however. We should be more concerned with the long-time production records of groups of cows rather than with unusual

records of scattered individuals. How far we can still go in this direction is indicated by the fact that on January 1, 1941, there were only 763,502 cows on test in 31,381 herds. This represents only slightly over 3 per cent of the cow population.

Further marked improvement activities in the development of the purebred business seems only natural. In addition to satisfying a "hankering" for owning purebred dairy cattle a real impetus seems to be present in the records of purebred herds in dairy herd improvement associations. Last year registered cows in D.H.I.A.'s produced 8,400 pounds of milk and 343 pounds of fat; grades produced 7,728 pounds of milk and 319 pounds of fat. These figures are based on 17,000 and 21,000 random samples of registered and grade records, respectively.

It has been estimated that there are about 175,000 registered sires in service. That's one registered bull for every 138 cows. At one time that would have seemed like an impractical and impossible ratio but with the coming of another milestone, artificial insemination, perhaps it is not. At the present time only one in ten of the purebred bulls born each year is registered. Some of those not registered would not be suitable herd sires but a great many of them are disposed of because there is no market for them. Steps to increase the demand for purebred bulls, together with proper use of artificial insemination, should result in more rapid advancement of the dairy breeds than has ever been possible in the past.

In the economic and agronomic phases of dairy production there have also been many milestones,—too numerous to discuss in detail by one not qualified to do so. The general trend in the economic field has been to reduce costs through more efficient production methods and to facilitate disposition of the product through cooperative effort. About 40 per cent of the milk leaving the farm is now handled by farmer cooperatives at some stage. Interspersed with more economical production procedures have, of course, been new equipment and machinery, new crops, new feeds, new fertilizers and fertilizer treatments, new methods of handling crops, of which ensiling has probably been of greatest importance, and systems of pasturing that have extended the grazing season through greater utilization of early spring and late fall pasture, and by the use of emergency crops to tide over dry periods.

Disease eradication is a field of activity in which the results are only too apparent. We are all familiar with the success of the tuberculosis eradication program. Area testing was completed on November 1, 1940. This meant that in this country bovine tuberculosis had been reduced to less than one-half of one per cent. That is the best record of any country in the world. The history of Bang's disease control is replete with startling discoveries and equally startling but usually ineffective remedies. It was in 1913 that the Vermont Experiment Station qualified for the Blue Book

because it was then that the advocator (Rich) of methylene blue as a remedy for Bang's disease was busily at work at that institution. The test and slaughter program has of course been very effective. Within a period of six years of Federal and State cooperation 346 counties in 20 states were in modified accredited Bang's disease-free areas, and 220 additional counties were under test. That represents progress and every effort must be exerted to see that adequate Federal and State appropriations are made to continue this work. Along with this program there is now the additional procedure of calfhooed vaccination which represents another forward step in disease control. The possibility of breeding Bang's disease resistant families is not to be ignored, in view of the progress that has been made in this direction with swine.

Mastitis is now our number 1 disease problem and offers a challenge to research that eventually will be met. We know pretty well how to prevent the spread of this disease from animal to animal, but how successfully to prevent or destroy the infection will be the aim of much talented effort.

The matter of housing was at first one of protection from bad weather. With our increased knowledge of sanitation and physiology many refinements have been made. The relationship between environmental conditions and physiological function is still vague, and perhaps when the facts are all known we will find that we have been pampering too much.

In discussing improvement in milk production it was intimated that proper selection and elimination of animals, as well as intelligent breeding, were responsible. Of course other factors were also at work, the chief one of which probably was improved feeding. Indeed, the phase of dairy production commonly included under feeds and feeding and nutrition affords a series of milestones that are rather well defined and, to one who has been working in this field, these naturally seem especially important. All will at least agree that no matter what inherited ability to produce a cow may have this will not be fully exercised unless she is properly fed.

The first attempt to make dairy cattle feeding something other than haphazard or inconsistent grew out of increased knowledge of agricultural chemistry and resulted in the formulation of feeding standards. Such names as Groven, Wolff, Lehman, Haecker, Kellner, Armsby, Savage, and Morrison are familiar to all students of dairy cattle feeding, for these are the ones who provided the basis for scientific feeding. Protein and energy are the only requirements to be met in a feeding standard and for a long time it was felt that nutritive ratio, an expression of the balance between digestible crude protein, digestible carbohydrates, and digestible fat, was the all-important thing. Balanced rations were the vogue. Now we know that a ration must be not only balanced but complete. Our present feeding standards are probably neither infallible nor final. Even now a new system is

being developed for evaluating feeds, using casein and glucose as reference materials.

Much work has been done on the protein requirement of dairy cattle with the trend being downward with respect to actual amounts needed. Whereas it was once common for grain mixtures to contain 24 per cent or more of total protein it is now common for grain mixtures to contain 12 to 16 per cent total protein and give as good results. This represents an enormous saving, since protein is the expensive ingredient of purchased feeds. The science of nutrition has shown what the end-products of protein digestion are and which of these amino acids are essential for some species. As yet, however, the amino acid requirement of cattle is not known and may be of little importance since the microorganisms of the rumen are capable of converting simple nitrogen compounds into complex proteins. Urea and ammonium salts may some day be important nitrogen sources for dairy cattle.

Digestibility and balance trials, feeding experiments, and net energy determinations have contributed much in rapid succession to our knowledge of dairy cattle nutrition, but no one of these was more important than the now famous Wisconsin experiment in which good results were obtained on a ration made entirely from the corn plant and disastrous results when the wheat plant only was used. The difference between these rations could not be detected by chemical means and suggested that the then current basis of formulating rations was inadequate. This experiment, the results of which were published in 1911, stimulated the use of purified diets with small animals, a procedure which resulted in the discovery of the first vitamin in 1913.

The discovery of vitamins certainly was another milestone in dairy production, although what at first seemed a very complex and critical problem has resolved itself, up to the present, into one of quality in roughages. We now know that dairy animals need the fat-soluble vitamins A and D in their feed, but the simple procedure of furnishing pasture and sunlight in the summer, sun-cured hay of high quality and some kind of silage in the winter, seems to satisfy the body needs for these factors, except possibly in the case of calves born in the fall and winter until such time as they are able to consume two or three pounds of hay.

Of the water-soluble vitamins, those comprising the vitamin B complex offer no problem since it has been demonstrated that thiamin, riboflavin, pantothenic acid, and pyridoxine are synthesized in the rumen. Vitamin C is probably synthesized somewhere within the body and cannot be said to offer a problem in dairy cattle feeding in spite of the beneficial effects on reproduction of vitamin C injections. Within the vitamin field itself demonstration of the remarkable rejuvenating effect of injecting vitamin C into bulls and cows suffering from some form of reproductive failure is one of

the most far-reaching contributions that has been made to economical production.

Vitamin E still affords a field for fruitful research, especially since alphatocopherol, the pure substance, is now readily available. Although some European work is quite persuasive as to beneficial effects on reproduction in cattle injected with vitamin E in the form of wheat germ oil, there has been no work on this continent that indicates the need for more vitamin E than is found in natural dairy feeds. This is not proof that additional vitamin E might not be beneficial, and in view of some favorable preliminary work at Iowa with ewes fed wheat germ oil by capsule we must withhold judgment and keep an open mind on this question.

Long before the vitamins were discovered the importance of certain constituents of ash was being demonstrated. Calcium and phosphorus were found to be the principal constituents of bones and their need was readily shown by restricting their intake. Later, their relationship to vitamin D became clear. What milestones they were when the first mineral balances with cattle were run and the actual requirements for calcium, phosphorus and other inorganic substances were determined in many successive experiments. The work with minerals, just as that with vitamins, has taught us the importance of good roughage and has prevented much of the exploitation of individual or "shotgun" mineral and vitamin preparations. At the same time the research has been broad enough to detect the conditions under which additional calcium, phosphorus, iodine, iron, copper and cobalt are needed.

For a long time the fat in feeds was looked upon only as a source of energy, yielding $2\frac{1}{4}$ times as many calories as the same amount of protein or carbohydrate. With the discovery of the fat-soluble vitamins and of the indispensability of certain fatty acids, the importance of fat assumed greater significance. This was climaxed by the demonstration that fat played some role in milk secretion. Today the amount of fat in dairy feeds is receiving the attention of research workers, farmers, and feed manufacturers.

We have seen many developments in feeds themselves. Processing of all kinds has been tried to improve the original value of feeds: cooking, steaming, enzymatizing, sprouting, chopping, grinding,—just to mention a few. As a rule it has been found that the cow herself is the best processor.

No discussion of the role of feeding in the development of dairy production would be complete without some mention of the commercial feed industry. Many dairy farms could not operate at a profit without having available when needed commercial feed mixtures to supply the nutrients and energy their own farms did not provide, and most dairy farms would not like the prospect of not having readily available a few individual feeds such as wheat bran and one or more of the oil meals. The manufacturers of commercial feeds have much to offer to the farmer and to those who are concerned with his education and improvement. It is gratifying to see the fine rela-

tionship that now exists between (reputable) commercial feed men and those in the educational field, including research. Further improvement of this relationship will prove mutually beneficial.

We now come to a consideration of the material—milk—for the production of which everything that has been said before has some significance. Oliver Wendell Holmes said once that “a pair of substantial mammary glands has the advantage over the two hemispheres of the most learned professor’s brain, in the art of compounding a nutritive fluid for infants.” That may have been true once but no one can now deny that our present knowledge makes it possible for use to provide the materials from which a better nutritive fluid for infants will be produced. Probably the first clue to this possibility came in 1905 when an investigator in Holland, after finding that the addition of milk to a diet of casein, albumin, rice flour, lard, and a mixture of all the then known essential inorganic salts, made the difference between life and death in mice, wrote “there is a still unknown substance in milk which, even in very small quantities, is of paramount importance to nourishment.” Should the writer, Pekelharing by name, be alive today and realize that his unknown substance was really a combination of now recognized vitamins, he would feel much like the piscatorial creature his name simulates.

For a long time the value of milk as a food was taken for granted. Consequently, emphasis was placed on improving the sanitary aspects of milk production. Can’t you just picture some of the old town meetings at which some patriarch would get up and say “We’re going to have clean milk in this town if we have to take the bull by the horns.” As a result of outbursts like this such great advances have been made in the sanitary aspects of milk production that the consumer in communities where milk control is exercised is practically assured of the safety of the milk he drinks.

As the science of nutrition was developed and new hitherto unknown factors that contribute to the value of foods were discovered, the value of milk as a food was reinvestigated. As a result of this newer knowledge of nutrition, many of the things formerly taken for granted have been shown to have sound scientific basis, and other favorable attributes not previously known have been brought to light. At the same time one or two weaknesses or deficiencies were encountered that made it possible better to understand the limitations of milk, as well as its virtues. Knowledge such as this regarding any food product results in more intelligent use of it.

It would be presumptuous for me to attempt to enumerate those factors in milk that make it “the most nearly perfect food,” other than to remind you that the concentration of certain vitamins in milk can vary several hundred per cent by employing special conditions such as the feeding of irradiated yeast to increase vitamin D and the use of grass silage for retaining summer vitamin A potency and esthetic appeal.

One approach to an evaluation of the nutritive value of milk as it is produced might be justifiable. There are eleven nutritional factors needed by humans concerning which quite definite information is available. These are calories, protein, calcium, phosphorus, iron, vitamins A and D, ascorbic acid, thiamin, riboflavin, and nicotinic acid. If the required amount of each of these eleven nutrients is considered as 1, and the amount of each nutrient furnished by a quart of milk is given a fractional part of 1, it will be found that 5.5 of the 11.0 units have been supplied.

This does not mean that two quarts of milk will satisfy all the dietary requirements, because at that rate of consumption calories, iron, and certain vitamins would still be short, and there would at the same time be a great excess of other factors, particularly calcium and phosphorus. It does point out strikingly, however, that because of the variety of nutrients furnished, liberal use of milk makes easier the selection of foods that are needed to round out the diet and make it complete.

The comparison just made was based upon milk as it leaves the cow. By the time that milk has been handled, processed, and handled again, some of its original nutritive properties have been lost. More detailed studies of these losses are needed and methods for preventing them must be devised. Otherwise we may some day find ourselves in the same predicament as the millers who after years of milling out some of the most valuable portions of the wheat kernel are now all enthused, with government encouragement, about putting those things back into flour.

That naturally raises the question of mineralization and vitaminization of milk. In a paper given at the Illinois meeting of the American Dairy Science Association in 1933, after justifying the fortification of milk with vitamin D, I made the following statement:

The general mineralization and vitaminization of foods, including milk, would further complicate an already complicated situation. Vitaminization and mineralization of foods probably cannot be justified except where natural foods fail to furnish these vital factors. This is especially true of milk. To add various vitamins and minerals to milk haphazardly would . . . jeopardize the unique and excellent position which this product now enjoys in the eyes of the general public and the medical profession. In spite of the intriguing mystery and glamor that surround some of the newer discoveries in nutrition we must not lose sight of the fact that plain, ordinary milk is the best single food we have and is thus considered by all. The fact that a sufficient intake of calcium cannot be obtained except by the inclusion in the diet of some form of milk or cheese places these dairy products on a pedestal by themselves.

I still believe that statement, knowing full well that good arguments to the contrary can be advanced.

Let us consider what possibilities there may be. I have here a series of vials, each of which contains a pure vitamin or the purest known form. With our knowledge of chemistry where it is, how simple it would be to add any or all of these to milk without impairing its flavor and possibly improv-

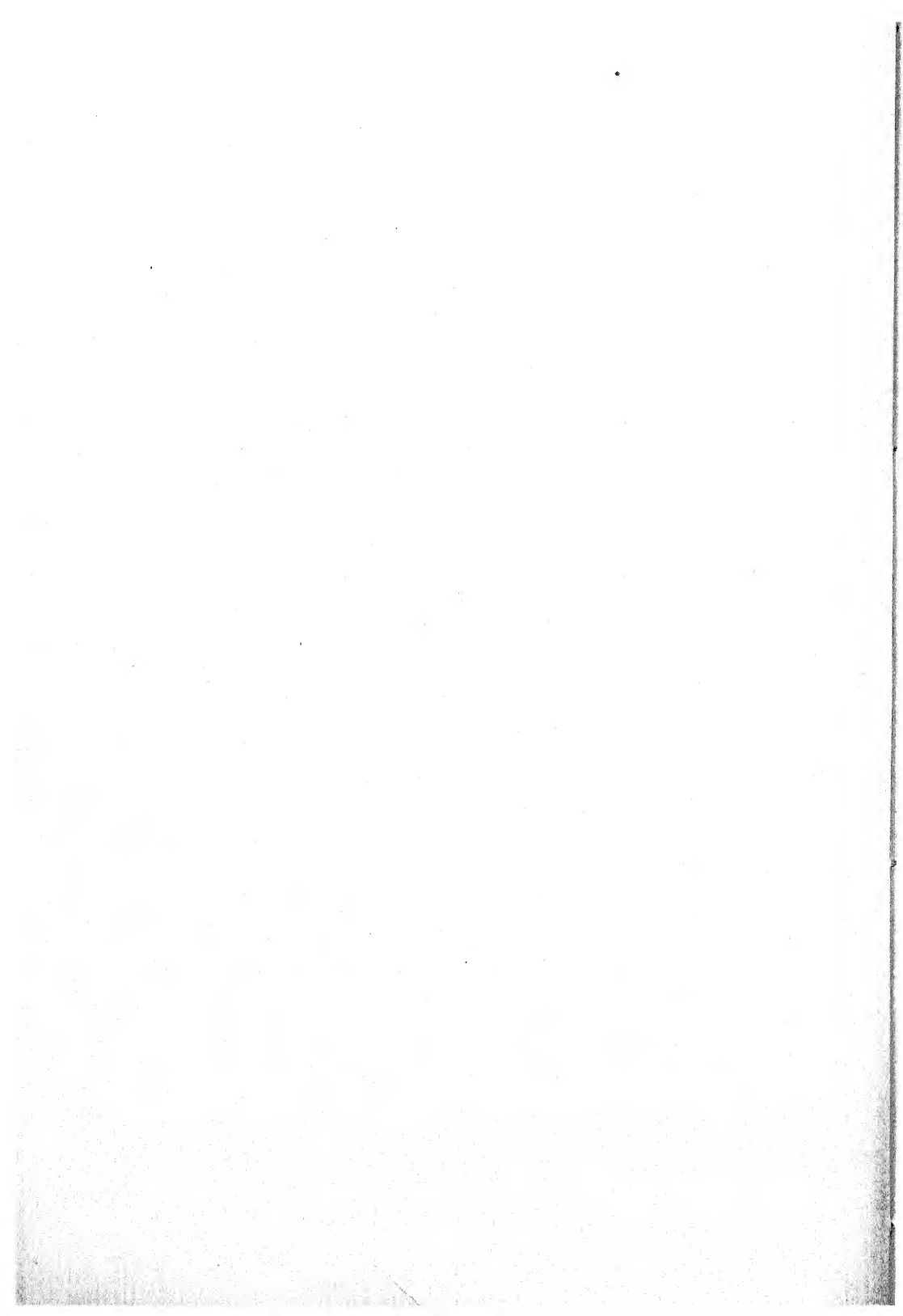
ing its appearance! Should promiscuous fortification of milk be employed the only purpose of feeding cows would be to produce pounds of milk regardless of its food value. Fortunately, however, the present trend of agriculture, plus knowledge gained by experience and research, leans towards systems of feeding that not only produce large quantities of milk but impart to that milk the highest nutritive value.

But why pay any attention to the food value of milk? Do you think for a moment that milk food value education is going to increase milk consumption in a hurry? Not at all, unless a carefully planned, high-class radio program on a national hook-up is used. Education at best is a slow process. If you really want to increase milk consumption reduce the price of milk to the consumer or increase the payrolls. The city of Akron, Ohio, is now at the top of the list with respect to per capita milk consumption. A year ago it wasn't. What happened? For one thing industry has picked up; for another, milk has been sold in gallon jugs for 7, 8, and 9 cents a quart. Take a look at evaporated milk. In 1921 canned milk consumed was 9 per cent of the liquid milk purchased; in 1939, canned milk constituted 13 per cent of the total purchases of milk, *i.e.*, fresh and canned combined. It is quite safe to speculate that even more evaporated milk would be consumed if the flavor were better. I am not advocating jug milk and evaporated milk; I am merely trying to illustrate what price will do.

This becomes more striking when we realize that in normal times nearly two-thirds of our families have incomes of less than \$1,500 and that the average income of these families is only \$826. Nearly 42 per cent of our families provide only 26 per cent of our food market. That is the explanation of the paradox of want in the midst of plenty. A study of the food purchases of families as related to income shows that the consumption of evaporated milk actually goes down while that of bottled milk goes up as income rises above \$750 a year. To what other conclusion can one come than that incomes must be increased or the price of milk reduced if the milk consumption level is to reach the point that seems best for conservation of our greatest natural resource—our people?

If there is a keynote to this address, it is this: economical production and, by inference, economical handling all along the line. I am amused at some of the reports that come to my desk as, for example, the one that listed the estimated price of all milk delivered to a particular market as \$2.00 per hundred and the cost of producing that milk, using the Michigan formula, \$1.98 a hundred. And yet many of the producers in that milk shed were making money because they were better than average. To raise the average we must have better land, better feed, better cows, and better men. It is our responsibility to help others attain all these things, and that probably means that in the future we will need to work more with the below average man than in the past.

As we meet here this week may we do so in a spirit of thankfulness that our work can be aimed at individual improvement rather than at the welfare of the State or party, for if the world wants to preserve science as a powerful social force for good the research man must be permitted to work without intellectual restraint, *i.e.*, he must be permitted to enjoy the fundamental freedom of democracy.



THE THIRTY-SIXTH ANNUAL MEETING OF THE
AMERICAN DAIRY SCIENCE ASSOCIATION

R. B. STOLTZ
Secretary-Treasurer

The American Dairy Science Association assembled in the gymnasium on the campus of the University of Vermont in Burlington on Tuesday, June 24, 1941, at 9:30 A.M.

The Honorable Warren R. Austin, Senior U. S. Senator and Trustee of the University, was introduced and delivered the address of welcome.

President Harry W. Cave then gave the following response:

"Senator Austin, I can assure you that we all appreciate very much your kind words of welcome, and the many things which have already been done to make our visit to Vermont pleasant and our meetings a success. Some of us felt that we were coming a long way from home when we came here but the cordial welcome we have received has made us forget that. I only wish that every member of our Association might be here to enjoy your delightful state and the many courtesies being shown us.

"We have come to New England for our thirty-sixth annual meeting because it is a definite policy of the American Dairy Science Association to hold its meetings at various rather widely separated points. In 1939 the annual meeting was held in the far West, then last year in the middle West and now in the East. This plan should make it possible for even somewhat isolated members to attend at rather frequent intervals.

"To further increase the benefits of the Association to its membership, there have been formed three branches of the organization known as the Eastern, the Western and the Southern Divisions. These divisions each hold an annual meeting with a program of contributed papers and a discussion of problems peculiar to the area concerned.

"Last year a committee was appointed from the Southern Division to prepare a history of that organization. This committee, consisting of J. A. Gamble, L. A. Higgins, C. A. Hutton, and J. A. Arey with W. E. Wintermeyer as chairman, presented their report at the annual meeting of that Division held in Atlanta, Georgia, on February 5-7, 1941.

"The Southern Division of the American Dairy Science Association had its inception at an informal conference in the hotel room of J. A. Gamble of Maryland during the National Dairy Show which was held at St. Paul, Minnesota, in October, 1921. Those meeting with Professor Gamble to discuss the proposed organization were C. W. Holdaway and F. A. Buchanan of Virginia, L. P. LaMaster of South Carolina, and C. A. Hutton of Tennessee. This group decided to make an effort to establish a Southern

Division and that letters concerning the proposition should be prepared, calling a meeting to be held during the Southern Agricultural Workers Conference scheduled for Atlanta, Georgia, on Feb. 20, 21, 22, 1922. The following letter prepared by J. A. Gamble and C. W. Holdaway was mailed to each dairy worker in the South on February 1, 1922.

Blacksburg, Va.
February 1, 1922

Subject: Meeting of Southern Workers in Dairying at the
Association of Southern Agricultural Workers,
Atlanta, Ga., February 20, 21, 22, 1922.

Dear Sir:

Arrangements have been completed for the meeting of the workers in dairying in the South for the purpose of forming a Southern Division of the American Dairy Science Association.

Every worker in dairying within the scope of this Division, which should include Maryland and West Virginia on the north, and Kentucky, Tennessee, Arkansas and Texas, and all States south and east of these, should be at the meeting. Every worker is an important factor in the dairy development of our Division and should emphasize his importance by being at this meeting.

The time and place will be on the afternoon of the 21st of February at the Piedmont Hotel.

A few short addresses will be given to emphasize the importance of this organized movement.

If for any reason you cannot be there, mail a letter to me at the Piedmont Hotel enclosing, first, your application for membership in the American Dairy Science Association if you are not a member already, and secondly, your vote on affiliation with the Southern Division when it is formed.

Very truly yours,

(Signed) C. W. HOLDAWAY,
Professor of Dairy Husbandry.

“On Feb. 3, 1922, J. A. Gamble, as chairman of the membership committee of the American Dairy Science Association, mailed a letter to the following dairy department heads:

H. E. Dvorachek, Arkansas	M. R. Tolstrup, S. Carolina
M. P. Jarnagin, Georgia	C. E. Wylie, Tennessee
J. J. Hooper, Kentucky	J. A. Clutter, Texas
J. M. Cadwallader, Louisiana	C. W. Holdaway, Virginia
L. A. Higgins, Mississippi	E. L. Anthony, West Virginia
R. H. Ruffner, N. Carolina	

“In this letter Professor Gamble called attention to the Atlanta meeting and enclosed blanks of application for membership in the American Dairy Science Association. A form letter containing information about the Association and an invitation to eligible dairy workers to join was also enclosed.

“ ‘As announced in the letter sent on Feb. 21 to all workers, the organization meeting of the Southern Division was held on Feb. 21, 1922.

“ ‘The following are the minutes of that meeting:

The first meeting of the southern members of the American Dairy Science Association was held at 4 p.m., in Room 911, Piedmont Hotel, Atlanta, Ga., Feb. 21, 1922. The members present included:

C. W. Holdaway, Virginia	J. A. Gamble, Maryland
C. E. Wylie, Tennessee	Stanley Combs, N. Carolina
M. P. Jarnagin, Georgia	L. H. Marlatt, Georgia

In addition to the members of the Association, J. P. LaMaster, South Carolina; J. M. Scott, Florida; R. C. Curtis, North Carolina; Dr. E. S. Good, Kentucky; J. C. Grimes, Alabama; C. E. McWhorter, Georgia, and several other leaders in the dairy and animal husbandry work in the Southern States were present.

The meeting was called to order by C. W. Holdaway of Virginia and J. M. Scott of Gainesville, Fla., was named temporary chairman. C. W. Holdaway was nominated and elected president; C. E. Wylie of Knoxville, Tenn., vice-president; and J. A. Gamble, College Park, Md., secretary-treasurer.

The first business of the meeting was to have the proposal explained and to pass a resolution asking that the executive committee of the American Dairy Science Association grant permission for the formation of a Southern Division of that body. Letters in support of such a proposal were read from several animal and dairy husbandry workers who could not be present.

It was moved that a committee of one be instructed to draft suitable by-laws and present them at the next meeting for discussion and adoption. J. A. Gamble was designated to bring in these suggested by-laws. It was further moved that as soon as the approval for the formation of the Division was received, C. W. Holdaway, the presiding officer, appoint the necessary committees—the committee list of the American Dairy Science Association to be used as a guide in the matter.

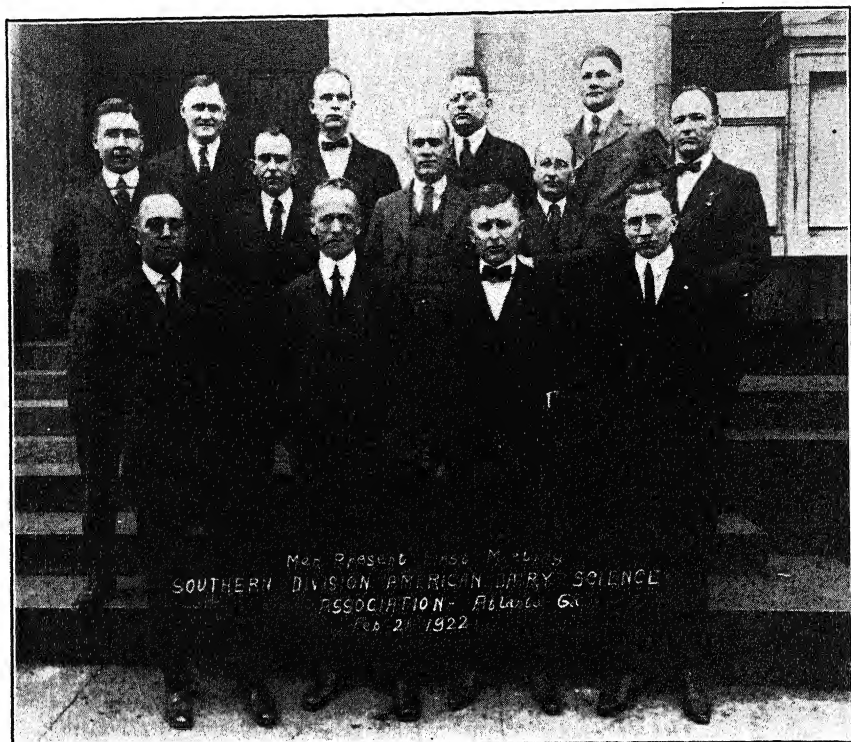
All present took part in the discussion relating to the opportunities for service of such a group in the improvement of dairy conditions in the Southern States. It was the general opinion that such an organization would not only result in the coordination of teaching, research, and extension work in the subject, but result in activities such as sectional dairy cattle and dairy products judging contests, to be held once a year at some central point.

The meeting of the group adjourned at 6 p.m.

(signed) J. A. GAMBLE,
Secretary-Treasurer.

“ ‘A second meeting was held the next morning, Feb. 22, 1922, at which the proposal to sponsor dairy cattle and dairy products judging contests was discussed. A committee was appointed to present the matter to the superintendent of the Southeastern Fair Association and report back.

“ ‘Arrangements were made for a picture of the newly organized group to be taken on the steps of the Carnegie library at 12:30 p.m. and copies of this picture have been preserved.



TOP ROW—E. L. Jordan, La.; S. Combs, N. C.; McWhorter, Central of Ga. R. R.; Rigdon, Central of Ga. R. R.

SECOND ROW—C. E. Wylie, U. of Tenn.; J. P. LaMaster, Clemson Coll.; J. F. Bazemore, Central of Ga. R. R.; E. S. Good, U. of Ky.; John M. Scott, Fla. Agr. Exp. Sta.

BOTTOM ROW—L. H. Marlatt, U. of Ga.; C. W. Holdaway, Va. Polytech. Inst.; J. A. Gamble, U. of Md.; L. J. Horlacher, U. of Ky.

“ ‘Under date of Feb. 24, 1922, J. A. Gamble, the newly elected Secretary-Treasurer, wrote President C. H. Eckles of the American Dairy Science Association requesting permission from the Executive Committee to organize a Southern Division of the Society. He enclosed twenty applications for membership.

“ ‘Favorable action on the part of the executive committee was reported by J. B. Fitch, secretary-treasurer of the Association, in a letter to J. A. Gamble dated March 16, 1922.

“ ‘The officers of the newly formed division arranged a program of papers and discussions for a regular meeting held Feb. 6 to 8, 1923. It is significant that the first subject under discussion was that of pasture grasses and pasture management for the South, presented by C. E. Piper, Forage Crops Division, U. S. Department of Agriculture. Attention given this

subject by early leaders of the Southern Division has helped to give it much needed impetus in the Southeastern states. Other papers presented at this and later meetings had to do with problems in teaching, feeding, breeding, etc., peculiar to the South. Each year a meeting is held during the annual convention of the Southern Agricultural Workers and the division has continued to grow in size and its activities.

“The chairmen who have served the Southern Division since C. W. Holdaway in 1923, have been:

C. E. Wylie, Tenn.	1924	R. B. Becker, Fla.	1933
J. P. LaMaster, S. C.	1925	A. D. Burke, Ala.	1934
C. A. Hutton, Tenn.	1926	R. H. Lush, La.	1935
J. S. Moore, Miss.	1927	E. C. Elting, S. C.	1936
J. S. Moore, Miss.	1928	A. H. Kuhlman, Okla.	1937
A. C. Baer, Okla.	1929	C. N. Shepardson, Tex.	1938
R. H. Ruffner, N. C.	1930	T. B. Harrison, Tenn.	1939
L. A. Higgins, Miss.	1931	C. G. Cushman, S. C.	1940
Earl Weaver, Okla.	1932	R. E. Waters, Miss.	1941

“The secretaries since J. A. Gamble, who served during the period 1923 to 1926, have been:

J. P. LaMaster	1927	A. H. Kuhlman	1935
A. C. Baer	1928	C. N. Shepardson	1936
L. A. Higgins	1929	T. B. Harrison	1937
J. S. Moore	1930	C. G. Cushman	1938
R. B. Becker	1931	R. E. Waters	1939
A. D. Burke	1932	C. D. Grinnells, N. C.	1940
R. H. Lush	1933	R. B. Becker	1941
E. C. Elting	1934		

“The object of our Association should be to serve dairy workers and the dairy industry as universally as possible. Our field of service has been expanded and our membership greatly strengthened through these branches of the parent organization, the Eastern, the Western, and the Southern Divisions.

“I would not close without expressing my sincere appreciation to the officers, the directors, the many committee members, and the individuals who have assisted in carrying on the work of the Association during the past year. Our organization is steadily growing and its work is constantly becoming more complex. The point has long ago been passed where this work could be done by a few officers. It has been a real pleasure to me to find the great willingness with which the many who were called upon, accepted the duties requested of them.

“We are now facing a future of great uncertainty. Our Association and our membership may be called upon for much greater contributions in the near future than in the past. With such hearty cooperation as has been

shown by our members in the past I have little fear but that they will give a good account of themselves whatever may come in the future."

President Cave then introduced Dr. W. E. Krauss, Associate in Dairy Nutrition Ohio Agricultural Experiment Station of Wooster, Ohio, who gave an address entitled, "Dairy Production Milestones," which will be found printed elsewhere in this issue of the Journal.

There were 298 members present. The meeting adjourned at 11:30.

GENERAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

Burlington, Vermont, June 26, 1941

President Cave called the meeting to order at 3:30 P.M. in the Fleming Museum, there being 124 present. Mr. Charles Blackman, chairman of the Necrology Committee, reported the death of the following members during the past year: Edward B. Meigs, A. R. Schubert, Elmer S. Hinman, Hugo Larsen, Clarence H. Redding, and Godfrey L. A. Ruehle. Information regarding the activities of these deceased members was contained in the report of the committee. Upon motion duly seconded the report was accepted to be made a matter of record in the minutes.

Editor Sutton then gave a report which will be found in the minutes of the board of directors.

MANUFACTURING SECTION

Secretary Anderson of the Manufacturing Section presented the following report:

The manufacturing section held its meetings at the scheduled hours and places. Mr. C. D. Dahle, chairman, presided.

All papers were presented as announced with the exception of "Production of Cream on Farms and in Plants," M 9, M 17, and M 30.

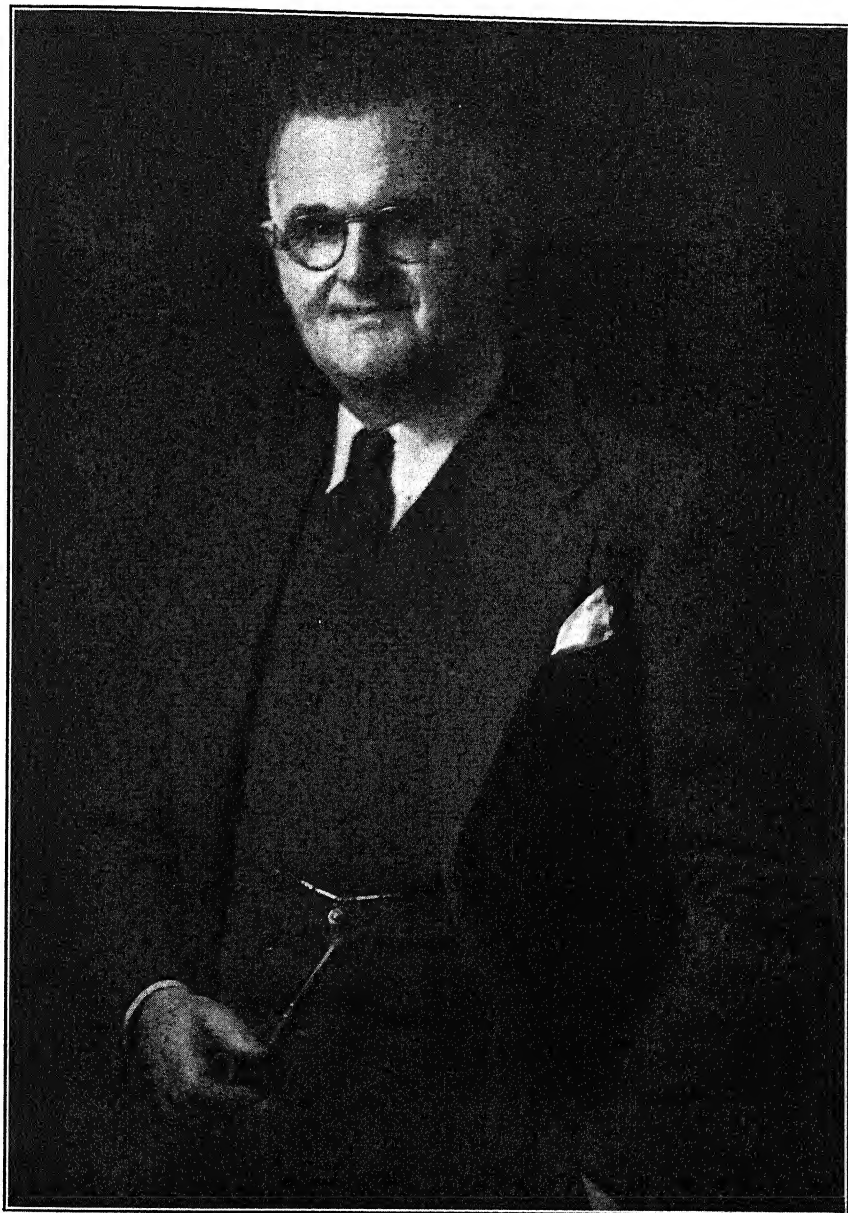
The business of the section was transacted at three meetings held at the announced places.

Reports were submitted by the various standing committees:

1. Committee on Chemical Methods for the Analysis of Milk and Dairy Products, L. C. THOMSON, *chairman*. The written report was accepted.

It was voted to appoint a special committee to compare the accuracy of the Gerber test with the Babcock test for milk and milk products.

It was voted that the chairman of the committee on the Chemical Analysis of Milk and Dairy Products act as coordinator in coordinating the work of said committee with like committees of other associations doing similar work. The report was accepted.



H. F. JUDKINS—PRESIDENT ELECT

2. Committee on Quality of Milk and Milk Products. W. V. PRICE in the absence of W. H. E. REID, *chairman*, called for sub-committee reports.
 - a. Cream quality—P. A. DOWNS, *chairman*; written report accepted.

b. Cream and butter quality—W. H. BROWN, *chairman*; written report accepted.

c. Ice cream—no report.

d. Cheese quality—W. V. PRICE, *chairman*; written report accepted.

e. Market milk—F. C. BUTTON reported for P. H. TRACY, *chairman*; written report accepted.

f. Condensed and milk powder—E. H. PARFITT, *chairman*; oral report accepted.

3. Committee on Students' National Contest in Judging of Dairy Products. G. M. TROUT, *chairman*; written report accepted.

4. Committee on Methods of Determining the Curd Tension of Milk. F. J. DOAN, *chairman*; written report accepted.

It was voted to accept the procedure for determining the curd tension of milk as recommended by the committee and that the committee be discharged.

5. Committee on Score Cards. C. J. BABCOCK, *chairman*; written report accepted.

It was voted to delete the flavor items under "remarks" on page 3 of the report and substitute words "flavor defects" listed on the other side.

It was voted that the score card for milk be approved as amended by the Manufacturing Section and submitted to the parent organization for final approval.

It was voted that the score card for ice cream be approved by the Manufacturing Section and submitted to the parent organization for final approval.

It was voted to accept the recommendations of the committee that cream be scored the same as milk until such time as a satisfactory score card can be devised.

It was voted that the committee remain active until it completes a score card for cream.

6. Committee To Study Methods for Measuring the Oxidation of Milk Fat. O. F. GARRETT, *chairman*; written report accepted.

7. Committee on Methods of Measuring the Color of Milk. O. F. GARRETT, *chairman*; written report accepted.

8. Committee to Study the Ways of Improving Summer Meetings of the Manufacturing Section. B. E. HORRALL, *chairman*; written report accepted. It was voted that the report of the committee be accepted and that the committee be discharged.

It was suggested that the retiring manufacturing chairman carry over as a fourth member of the program committee.

It was voted to appoint a committee of three to work with the present chairman of the section to organize the present complex committee slate, to be effective October 1, 1941.

The following officers of the Manufacturing Section were unanimously elected:

Vice-Chairman—R. WHITAKER

Secretary—KENNETH G. WECKEL

Mr. L. H. Burgwald, present vice-chairman, automatically becomes chairman of the Manufacturing Section.

Respectfully submitted,

(Signed) E. O. ANDERSON

Secretary, Manufacturing Section

Upon motion duly seconded the report of the Manufacturing Section was accepted and ordered to be printed in the minutes. Copies of the written reports of the various committees of the Manufacturing Section were filed with the Secretary for their preservation.

EXTENSION SECTION

Mr. J. F. Kendrick, Secretary of the Extension Section presented the following report:

In session at the University of Vermont, Burlington, Vermont, June 24th, 25th, and 26th, 1941.

The annual meeting of the Extension Section was called to order by the chairman, Otto J. Hill, June 24 at 1:30 P.M. in Morrill Hall. Forty-six members and 25 guests were present from a total of 25 different states.

During the three-day session the various committees of the Extension Section presented their reports supplemented with selected papers on pertinent phases of the dairy extension program.

The Sire Committee's report was presented by the committee chairman, E. J. Perry. The committee approved new age-conversion factor for use in Dairy Herd Improvement Associations; approved the use of Dairy Herd Improvement Association records in selective registry and Star Bull programs of the American Jersey Cattle Club; and recommended uniform methods of compiling annual reports of artificial breeding associations; also approved a bull leasing plan to combat the "stock-yard" bull problem.

Report was accepted.

The Feeding Committee report was presented by Mr. V. L. Gregg. This committee re-emphasized the importance of roughage feeding and outlined a tentative program to be followed by the Feeding Committee in succeeding years. The report was accepted as a progress report.

Quality Committee report presented by Mr. Evert Wallenfelt. The committee outline objectives of quality work to be discussed in future years. Report accepted as a progress report.

The Herd Health Committee reported to the joint session of the Extension and Production Section by Mr. C. G. Bradt. Report accepted as a progress report.

Report of the Joint Committee on Feeding Standards accepted.

Testing Committee report presented by Mr. C. R. Gearhart. The committee reported on standards for supervision of dairy herd-improvement association testing and recommended that further study be given the subject. The report was accepted as a progress report.

Type Classification Committee report given by Mr. J. W. Linn. The committee recommended type classification in herds where all cows are under test. Report accepted.

Exhibit Committee report presented by Mr. C. J. Fawcett. Exhibits illustrating extension methods used in 16 different States were presented.

Resolutions Committee report given by Mr. A. I. Mann, chairman of the Committee. The report was accepted and turned over to the General Resolutions Committee.

During the business session of the Section, Mr. E. C. Scheidenhelm of Michigan was elected Secretary. The officers who will assume their responsibilities on October 1, 1941, are as follows:

GLEN W. VERGERONT, *Chairman*

J. F. KENDRICK, *Vice-Chairman*, and

Chairman of the 1941 Program Committee

E. C. SCHEIDENHELM, *Secretary*

Respectfully submitted,

(Signed) J. F. KENDRICK

Secretary, Extension Section

Upon motion duly seconded the report was accepted and ordered to be printed in the minutes.

PRODUCTION SECTION

The Production Section held five regular scheduled sessions. Of these, two were symposia combined with the Extension Section and the Manufacturing Section respectively; a third was a Section symposium. The remaining two were devoted to regular papers and discussions grouped as to special subject matter. In accordance with the recommendation of the General Program Committee, this Section this year tested the plan of dividing the group into two divisions, with programs running concurrently. W. E. Petersen, Section Chairman, presided at sessions of Division A; H. A. Herman, Section Vice-Chairman, presided at sessions of Division B.

All sessions were well attended. Thirty-nine of the forty papers scheduled were presented.

The Section held three business meetings. Chairman W. E. Petersen presided at each meeting.

The minutes of the 1940 Annual Meeting of the Production Section at Lafayette, Indiana, were read and approved.

Reports of the various standing committees were submitted and ap-

proved. Copies of these reports are attached. Salient points incorporated in these reports and presented herewith to the General Session for approval are:

1. Breeds Relations Committee. W. T. CRANDALL (New York), *chairman*.

The revision of Rule 7 concerning the supervision of official tests will read: "Where the cows are milked by hand, only one cow may be milked at a time, if in a box stall or individual quarters. Two, however, may be milked at the same time, if milked by machine, or if standing in the stanchions in close proximity and in full view of the supervisor."

2. Committee on Measuring Results of Pasture Investigations. G. BOHSTEDT (Wisconsin), *chairman*.

This committee has continued its efforts of reconciling the viewpoints of workers engaged in pasture research. It is at present concerned with ways and means for printing their latest compilation of methods of pasture investigation technique.

3. Committee on Standard Methods of Analyses. W. E. PETERSEN (Minnesota), *chairman*.

Dr. Petersen stated that due to the large amount of material accumulated by the committee in the various phases of its study, it was deemed impractical to present the combined recommendations in a single report. The committee recommended that in view of the fact that methods of analyses by virtue of constant research were subject to frequent revisions and since the various fields had been carefully canvassed to date, it be discharged as a standing committee. It is further recommended that occasional papers be prepared for publication as reviews in the JOURNAL OF DAIRY SCIENCE, on standard methods of analyses in individual areas of dairy production research.

4. Committee on Rules for Conduct of the Students' National Dairy Cattle Judging Contest. I. W. RUPEL (Wisconsin), *chairman*.

5. Committee on Awards for Students' National Contest in Judging Dairy Cattle. A. A. BORLAND (Pennsylvania), *chairman*.

In addition to the regular awards that have been made at recent contests and that were repeated at the 1940 contest, two scholarships were secured. The Holstein scholarship of \$500.00 was provided by Mr. Forry Laucks, owner of Lauxmont Farms, Wrightsville, Pa. The Ayrshire scholarship of \$525.00 was provided by a group of prominent Ayrshire breeders in Pennsylvania.

6. The Feeds Specifications Committee. E. S. SAVAGE (New York), *chairman*.

1—The American Dairy Science Association shall prepare through its Feeds Specifications Committee, an official table of analyses of feeds for dairy cattle. This table shall contain the usual percentages of water, min-

eral matter, protein and total digestible nutrients. A column showing the therms of net energy shall be given. The digestion coefficients shall be given as determined by cattle whenever possible. Whatever is available as to mineral and vitamin content shall be given.

2—This table shall be revised annually as new values appear.

3—Referees for different feeds shall be invited by the feeds specifications committee to study and report on single feeds or groups of feeds. These referees shall report annually on changes in analyses and new discoveries with respect to other qualities. Referees shall automatically become ex-officio members of the feeds specifications committee.

4—Efforts shall be made to refine feeding values of feeds by stimulating feeding experiments.

5—The members of the Feeds Specifications Committee shall cooperate fully with feed control officials and with similar committees of the American Society of Animal Production.

A change in the method of the appointment of committees was voted, whereby the incoming Section Chairman was delegated to make such appointments for the ensuing year. It was voted also that all new officers take office immediately following the close of the present annual meeting of the Association. It was felt that such a practice would enhance the formulating of the program for the following year's meeting.

By audible expression and show of hands, the Production Section went on record in hearty recommendation of the plan of program for the present meeting and recommended the continuation of such type of program.

Several excellent suggestions were made for symposia topics for future meetings.

Professor J. C. Knott, chairman of the nominating committee, presented names of candidates for offices of vice-chairman and secretary of the Section for 1941-42. K. L. Turk, Maryland, was elected vice-chairman; and Dwight Espe, Iowa, was elected secretary. H. A. Herman, Missouri, vice-chairman for 1940-41, automatically becomes chairman.

Respectfully submitted,

(Signed) K. S. MORROW

Secretary, Production Section

The following list of committees for the Production Section for 1941-42 was named by Section Chairman H. A. Herman and submitted to the secretary following the reading of the foregoing report:

1. *Breeds Relations Committee:*

F. W. ATKESON, Kansas, *Chairman* (1 year)

H. A. HERMAN, Missouri, *Secretary* (2 years)

FLOYD JOHNSTON, Iowa (1 year)

W. W. YAPP, Illinois (2 years)

J. B. FITCH, Minnesota (3 years)
E. C. SCHIEDENHELM, Michigan (3 years)

2. *Committee on Measuring Results of Pasture Investigations:*

G. BOHSTEDT, Wisconsin, *Chairman*
R. H. LUSH, District of Columbia
I. R. JONES, Oregon
R. E. HODGSON, Washington
C. B. BENDER, New Jersey
R. B. BECKER, Florida

3. *Committee of Standard Methods of Analyses:*

Committee excused.

4. *Committee on Rules for Conduct of Students' National Dairy Cattle Judging Contest:*

I. W. RUPEL, Wisconsin, *Chairman*
S. M. SALISBURY, Ohio
J. R. DICE, North Dakota
E. M. HANSON, Iowa
P. M. REAVES, Virginia

5. *Committee on Awards for Students' National Contest in Judging Dairy Cattle:*

A. A. BORLAND, Pennsylvania, *Chairman*
BURT ODERKIRK, Babson Co., Illinois
G. E. TAYLOR, New Jersey
I. W. RUPEL, Wisconsin

6. *The Feeds Specifications Committee:*

(From the Production Section)

E. S. SAVAGE, New York, *Chairman* (2 years)
G. BOHSTEDT, Wisconsin (1 year)
C. D. GRINNELLS, North Carolina (3 years)

(From the Extension Section)

C. L. BLACKMAN, Ohio (2 years)
W. T. CRANDALL, New York (1 year)
M. J. REGAN, Missouri (3 years)

(Sub-committee on Digestion Coefficients)

F. B. MORRISON, Cornell, *Chairman*
W. E. KRAUSS, Ohio
S. BRODY, Missouri

7. *Committee on Silage Methods, Evaluation, etc.:*

C. B. BENDER, New Jersey, *Chairman*

T. E. WOODWARD (U.S.D.A.)

J. G. ARCHIBALD, Massachusetts

G. BOHSTEDT, Wisconsin

C. F. MONROE, Ohio

J. C. KNOTT, Washington State College

Upon motion duly seconded the report was accepted.

Mr. J. M. Frayer, Chairman of the Registration Committee, reported the attendance of 844 men, women, and children. Of the 492 men registered, 325 were active members and 167, non-members. These members represented 40 of the United States and Canada and the Philippines.

RESOLUTIONS COMMITTEE REPORT

Mr. K. S. Morrow, Chairman of the Resolution Committee, presented the following report:

The American Dairy Science Association assembled in its 36th Annual Meeting at the University of Vermont, wishes to express for the membership, their families and guests, its appreciation for the hospitality, delightful entertainment and splendid facilities provided by the officials and faculty of that University.

Therefore, be it *Resolved*: That the membership of the Association publicly express its most sincere appreciation to Dean and Director J. L. Hills; to Professor H. B. Ellenberger and his departmental staff; to the Ayrshire Breeders' Association, The American Guernsey Cattle Club, The Holstein-Friesian Association of America, and the American Jersey Cattle Club; to the several Vermont Maple Sugar producers and marketing organizations; and to all other agencies cooperating in the providing of entertainment and the many fine courtesies.

WHEREAS: The general health and physical well being of our people constitute the first essential in our national defense, and,

WHEREAS: The Selective Service Administration is finding an alarming proportion of our young men to be unfit for military service by reason of nutritional defects, and,

WHEREAS: Local, State and Federal health officials have long recognized the importance of an increased consumption of dairy products as a means of promoting national health and have encouraged and assisted the dairy industry in developing programs for the encouragement of increased dairy products consumption, and,

WHEREAS: There is an enormous potential supply of milk which can be developed whenever price and demand are sufficient to justify more liberal feeding of our dairy herds, and,

WHEREAS: The Government can always buy on the open market any needed supplies for shipment to Great Britain, and with this increased demand, effect an automatic adjustment of domestic supply and demand through resultant price changes without discouraging the desire for dairy products.

Therefore, be it *Resolved*: That this Association express its disapproval of any National program specifically designed to discourage the home consumption of dairy products or to develop in the minds of the consuming public the idea that dairy products are non-essential or unimportant in the National diet, which idea is in direct conflict with the long established and generally recognized recommendation of all public health and nutritional authorities, and,

Be it further *Resolved*: That a copy of this resolution be forwarded to the Honorable Secretary of Agriculture for his information.

WHEREAS: There has been a most valuable contribution to the National economy of this country through the educational and research activities of our Federal government, colleges and universities, and,

WHEREAS: The future welfare of the nation will depend to an even greater extent on activities of these agencies, and,

WHEREAS: The present emergency is necessitating the closest scrutiny and most conservative use of public funds and resources.

Therefore, be it *Resolved*: That this Association urge upon all public officials charged with the distribution of public funds, the importance of the continuance of an advancement of this program, and,

Be it further *Resolved*: That copies of this resolution be forwarded to the Honorable Secretary of Agriculture and all State Directors of Experiment Stations for their information.

WHEREAS: This American Dairy Science Association recognizes the valuable contributions made by the pioneer workers in doing research.

Therefore, be it *Resolved*: That this Association give public recognition to the excellent work developed by Dean J. L. Hills in the problem of experimental research methods and for his many other contributions to the field of Agriculture through his long years of valuable service as an instructor and Dean of Agriculture at the University of Vermont.

WHEREAS: The problems of herd health can best be attacked with the assistance and cooperation of all agencies and organizations concerned,

Therefore, be it *Resolved*: That a Herd Health Committee be appointed by the President of this Association to seek the cooperation of the American Veterinary Medical Association and the United States Live Stock Sanitary Board in formulating organized plans for an action program on these problems.

WHEREAS: This Association recognizes the importance of awards in giv-

ing incentive to students throughout the country for continued activity and study in the field of dairy cattle breeding and production,

Therefore, be it *Resolved*: That donors of prizes for the winners in the National Collegiate Students' Dairy Cattle Judging Contest be thanked individually in the name of the Production Section of the American Dairy Science Association by the Chairman of the Committee on Awards.

Be it *Resolved*: That the Dairy Cattle Breed Associations be commended for their constructive action in the organization of the Pure Bred Dairy Cattle Association of America, through which greater unification of plans and methods for the improvement of the various breeds may be attained.

WHEREAS: The Borden Company is continuing its awards for recognition of superior research in dairying.

Therefore, be it *Resolved*: That the American Dairy Science Association express its appreciation to the Borden Company for its continuing interest in dairying.

Respectfully submitted,

K. S. MORROW, *Chairman*

C. Y. CANNON

HAROLD MACY

E. C. SCHEIDENHELM

C. N. SHEPARDSON

Upon motion duly seconded the report was accepted.

NOMINATING COMMITTEE

Mr. Earl Weaver, Chairman of the Nominating Committee, submitted the following report:

For *Vice-President*: H. P. DAVIS; JAMES W. LINN

For *Director* to succeed M. E. Parker: L. S. PALMER; G. M. TROUT

For *Director* to succeed J. W. Linn: J. C. KNOTT; L. P. LAMASTER

Committee:

EARL WEAVER, *Chairman*

R. B. BECKER

R. R. GRAVES

J. B. FITCH

D. R. THEOPHILUS

Upon motion duly seconded the report was accepted.

SECRETARY-TREASURER'S REPORT

The Secretary-Treasurer then gave the following report:

The policy of the American Dairy Science Association in conducting their business has been changed in recent years, and at present the Board of Directors, who are elected for a period of three years by ballot, conduct

the business of your Association. It is their wish, however, that a summary of their action be presented to the membership so that the members present at this annual meeting may know as early as possible the action that has been taken by the Board of Directors.

A copy of the Certified Public Accountant's Audit was made February 1, 1941, and mailed to each member of the Board of Directors.

Although you may not be interested in a detailed report, you will be interested in learning that our income last year was \$17,347.00 and our operating expenses were \$16,542.00. Our net worth is \$18,919.55.

Our increased expenditures are largely due to increased cost of our Journal. Up until 1933 our Journals were limited to 550 pages per volume. In 1932 the style was changed so that each page contained an equivalent of one and one-third pages. In 1937 the volume of the Journal contained over 1100 pages which was twice as many pages as the volumes which had been published previous to 1934. Last year our Journal contained 1662 pages or more than three times the previous limit. When one takes into account both the style change and increased number of pages, the XXIII Volume (1940) contained four times as many words as did the volumes previous to 1932.

Our circulation reached 2438 in 1939 and 2406 in 1940, but on June 17 of this year we had 129 greater circulation than at the same time last year which would indicate that our circulation will exceed 2500 this year. This is 50 per cent higher circulation than in 1936 when it was 1652.

Our increased circulation this year is largely due to an increased number of student affiliates. Last year we had a total of 282. At this same date last year we had 242. We now have 368 which is an increase of 126 over last year. Of the 368 student affiliates, Iowa leads with 62. Ohio is second with 43; Wisconsin third with 26; Massachusetts fourth with 22; Illinois fifth with 17. Other states with ten or more student affiliates are Texas, Vermont, Pennsylvania, Indiana, Michigan, Missouri, New York, South Carolina, Virginia and the State of Washington.

New Members

We may expect to lose about 7 per cent of our membership each year by death, resignations or one thing or another. It is therefore essential that each state have new members to the extent of about 7 per cent of their membership to prevent a decreased number of members.

Up to this date this year we have 83 new members; 31 of whom were student affiliates and 52 of whom have paid the \$5.00 affiliation fee. Illinois and Pennsylvania lead with 7 new paid members each; Oklahoma, Vermont, and New York tie for third place with 4 each; and Massachusetts, Ohio, and Wisconsin tie for sixth place with 3 each.

Permit me to say a word about the affiliation fee. Some of our members

may not see the need of charging this fee. Our net worth, most of which is invested in United States Government Securities, amounts to \$18,919.55. If our 1400 members were to share this equally, each one of us would receive \$13.51. It is therefore only proper that these new members who have not contributed anything to this accumulation should pay \$5.00 or less than one-half of a membership's value into this fund.

This does not apply to student affiliates. They not only are permitted to receive the Journal at \$3.00 per year, which is less than our cost of printing the Journal, but upon finishing school, they are eligible to become full members by merely paying their \$5.00 dues. In 1939 we had 156 student affiliates; in 1940, 282; and this year we have 368 student affiliates.

Back Copies

The Association is now prepared to furnish to any of its members or any library a complete file of all back numbers and volumes. We suggest that you check your library and complete your volumes of the JOURNAL OF DAIRY SCIENCE. As soon as the twenty-year index is published the first twenty volumes will be a very valuable reference for your office and library. You will find the price list for back copies printed in the advertising section of the Journal.

Advertisers

We are grateful for the commercial companies that use our Journal as an advertising medium. Last year income for advertising amounted to over \$4,000, which is equivalent to the dues of 800 members. Any courtesies shown these advertisers will be appreciated.

Reprints

We recommend that those of you who are in charge of having bulletins printed that you investigate purchasing reprints through our Printers of all articles published in the JOURNAL OF DAIRY SCIENCE. We are of the opinion that the reprints will be furnished you at a lower cost than you will be able to have them printed, and the Association gets a small income from all reprints sold.

The Secretary then reported on all the action taken by the Board of Directors. Motion was made, duly seconded and passed that the Minutes of the Board of Directors be accepted and the Association approve and endorse all action that the Board of Directors had taken during the past year.

MEETING OF BOARD OF DIRECTORS AMERICAN DAIRY SCIENCE ASSOCIATION

R. B. STOLTZ, *Secretary-Treasurer*

Burlington, Vermont, 9:30 A.M., June 23, 1941

A meeting of the Board of Directors of the American Dairy Science Association was held in the Delta Delta Delta House, Monday, June 23, 1941, at 9:30 A.M.

Present: President H. W. Cave; Vice-President H. F. Judkins; Secretary-Treasurer R. B. Stoltz; Directors, J. W. Linn, M. E. Parker, C. N. Shepardson, Fordyce Ely, H. B. Ellenberger, A. C. Dahlberg, E. S. Guthrie.

Editor T. S. Sutton then presented the following report:

EDITOR'S REPORT

The Editor begs to submit the following brief report to the Board of Directors of the Association:

1. *Summary of Journal Contents.*

A summary of the Journal contents over the past three years is presented in the accompanying table.

SUMMARY OF JOURNAL CONTENTS

	1938-39	1939-40	1940-41
Number of original articles	88	94	97
Pages of original articles	760	826	892
Number of reviews	2	3	5
Pages of reviews	26	119	144
<i>Miscellaneous</i>	140	98	157
Students National Contest, Proceedings Annual Meeting, Announcements, Circulation, Index, Committee reports.			
Pages of Abstracts	232	206	304
Total number of pages printed	1158	1245	1497
<i>Classification of Articles</i>			
Manufacturing articles	52	53	56
Pages occupied by Manufacturing	482	450	514
Production articles	28	31	35
Pages occupied by Production	212	296	316
Manufacturing-Production	8	10	6
Pages occupied	66	80	62
<i>Classification of Reviews</i>			
Manufacturing Reviews			5
Pages occupied by Manufacturing Reviews			144

2. *Abstracts.*

You will note a substantial increase in the number of pages of abstracts

during the past year. We believe also that the quality of the work done has been improved. Steps have recently been taken to make further improvements particularly in reference to uniformity in the citation.

3. *Style Standard.*

The committee on Style Standard has made their report to the Editor and Journal Management Committee. A "Note to Contributors" has been prepared which we trust will be mutually helpful to author and editor. It is intended that this shall be printed on one Journal page in small type and regularly carried in the Journal.

Again we want to take this opportunity to publicly express our gratitude to all those who have given so generously of their time and energies in our assistance. To them is due the credit for any measure of success attained.

The following action was taken by the Board of Directors:

1. The Editor's report was accepted.
2. The Secretary was instructed not to publish a list of the members in the December Journal.
3. The budget was made for a period of 18 months.
4. Student branches were authorized at Cornell and Oklahoma.
5. From the report of the Committee on Journal Management:
 - A. The twenty-year index is to be sold at cost, which is approximately \$4.50. Pre-publication price to members is to be \$1.00; after-publication price, \$2.00. The Secretary was authorized to place the pre-publication price on annual statements.
 - B. Approve policy adopted last year in paying for abstracts.
 - C. Abstracts of papers presented at annual meeting are to be published in the August Journal.
 - D. The report of the Committee on Style Standards was adopted and ordered printed.
 - E. The pages per volume of the Journal should not exceed the present number.
6. Report of Auditing Committee was accepted.
7. The Secretary-Treasurer was authorized to send a copy of the minutes of the Board of Directors to each officer and director, and to condense the minutes that are to be printed into about 15 pages. He was further authorized to make a very brief report of all board action at the General Business Session.
8. The following report of the Committee on Divisions was accepted:
 - A. That each of the three divisions be paid \$25.00 upon receipt of a report of their meeting, and the names of the elected officers for the ensuing year.

- B. That the divisions hold their election by ballot of those members who attend the divisional meetings.
- 9. That the \$1,025 collected by Professor Borland be turned over to the Chairman of the Awards Committee of the Production Section when requested.
- 10. Texas was selected for the 1943 meeting.
- 11. The tentative program of the next annual meeting is to be printed separate and distributed with the May issue of the Journal.

AMERICAN DAIRY SCIENCE ASSOCIATION PRESENTED
BORDEN AWARDS

TO

P. F. SHARP

AND

E. B. HART

University of Vermont

Burlington, Vermont, June 26, 1941

Mr. H. B. Ellenberger acted as toastmaster at the Annual Association Banquet, and presented Gov. William H. Wills of the State of Vermont, Dean J. L. Hills of the University of Vermont's Agricultural College, and Mr. W. D. Dotterer, acting chairman of the Committee for the Borden Award in Dairy Manufactures.

Mr. Dotterer made the following statement:

"Corporations are not always the soulless organizations they have been accused of being. There are times when they show more humanitarian characteristics than some of the individuals who make the unwarranted accusations. This meeting is the result of far-sighted scientific appreciation by one of the large corporations engaged in the dairy business. They are under no obligation to industry or to the universities to provide a prize for outstanding work in dairy investigation or development, but they have generously provided two \$1,000 awards for such effort. May I take this opportunity to express the appreciation of the American Dairy Science Association to the Borden Company for this generosity in providing this very substantial gift to one of our great scientists.

"I do not suppose that any of the scientists engaged in dairy research have been influenced in the quality or quantity of their work by the thought of the Borden Award. Rather they pursue their labors with the object of learning the truth and making their work available to their colleagues and to the whole industry. If this statement seems doubtful, one need only note the number of new and improved processes which have been given to any who would use them for the benefit of humanity.



PAUL FRANCIS SHARP

"The man chosen to receive the award in dairy manufacture this year is well known to every one interested in the scientific aspects of dairying. He has been the inspiration and leader in a great number of important

researches. He is recognized as one of the best dairy scientists in the world. He is one of the quiet, unassuming, gentlemanly personalities whom it is a delight to know and to work with. His statements are carefully made and can be taken for facts. All I have said can be applied to many other investigators in the dairy field. In fact, the first impression made on the Committee when the data on so many men was presented for consideration was that a choice would be well night impossible. However, after much deliberation, a choice has been made. The Committee hopes and believes that out of the galaxy of stars a selection has been made with which you will agree. We know the successful nominee is worthy of the prize and only regret that more prizes were not available.

"It is not my duty to make a speech but to name the recipient of the Borden Award in Dairy Manufactures for 1941. He is Paul Francis Sharp of Cornell University. Dr. Sharp received his Bachelor of Arts degree at Nebraska Wesleyan University in 1917, his Master of Science at the University of Minnesota in 1920 and his Doctor of Philosophy at the University of Minnesota in 1922. He was student assistant in Chemistry at Nebraska Wesleyan and the University of Minnesota 1917-1922; also in chemical warfare service, U.S.A., 1918. He was associate chemist at Montana Agricultural Experiment Station 1922-25. He has been Professor of Dairy Chemistry and chemist in the Agricultural Experiment Station of Cornell University since 1925.

"Dr. Sharp is one of those tireless workers who have given so much to the Dairy Industry. His publications are legion and the information he has furnished has been of inestimable value commercially. It is not easy to say which of his accomplishments have been of the greatest use to dairying and to humanity. Studies on the lipolytic activity of milk in relation to flavor, studies on Vitamin C, studies on oxidized flavor, studies on the physical state of milk fat and studies on the de-aeration of milk are some of the projects which have helped to make him so well known.

"The Committee unanimously chose him as the most deserving and it is my privilege to present to you, Mr. Wentworth, Dr. Paul Francis Sharp, in order that you may give him the really substantial part of the award."

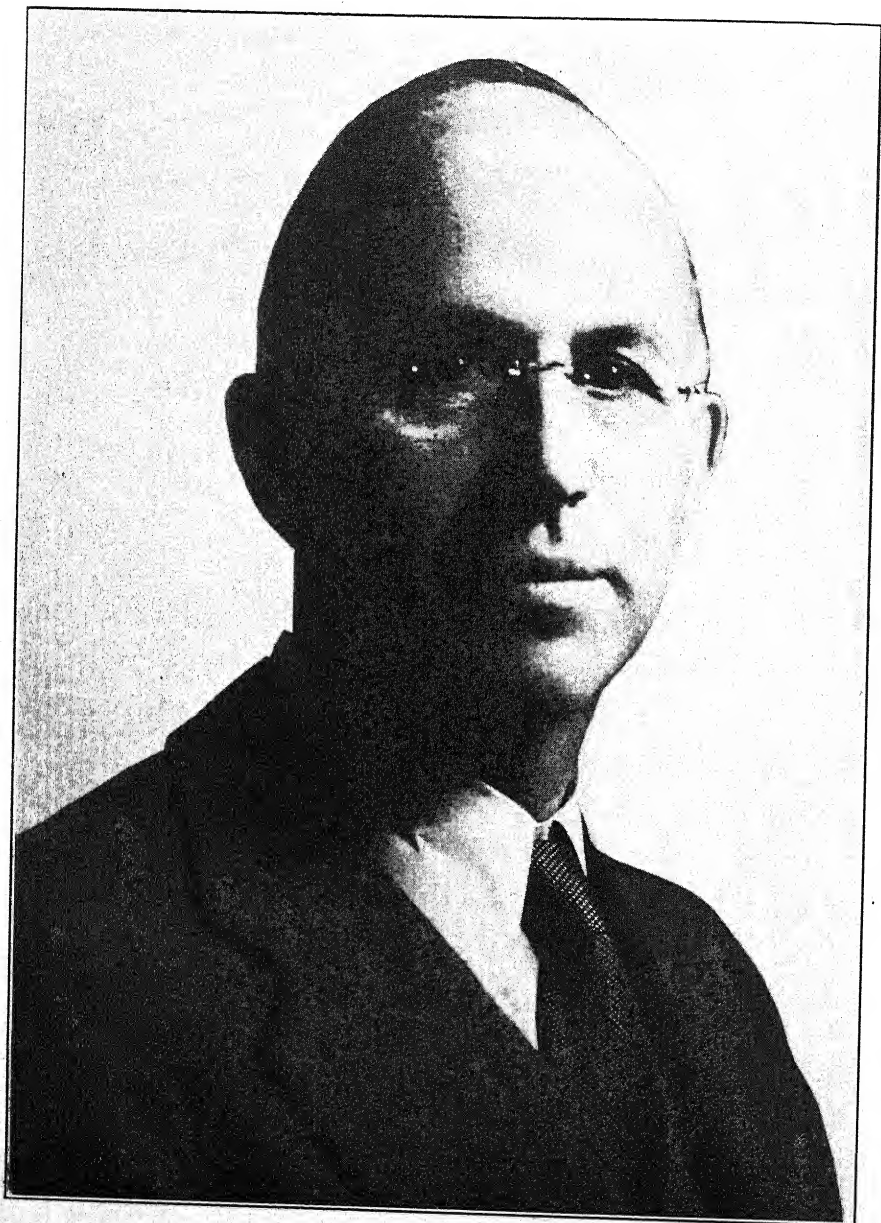
Mr. Sharp came to the platform, and Mr. W. A. Wentworth of the Borden Company presented Mr. Sharp a gold medal and a check for \$1,000.

Mr. Ellenberger, the toastmaster, then introduced Mr. G. C. White, acting chairman of the Committee for Borden Award for Production. Mr. White then made the following statement:

"The three members of the Production Award Committee, whose duty it was to select the recipient of the 1941 Borden Award for outstanding research in dairy production, have unanimously chosen Professor Edwin Bret Hart of the Wisconsin College of Agriculture for this honor. Professor

Hart's numerous contributions to our scientific knowledge and his service to the dairy industry over the last 40 years mark him as the outstanding candidate for this award.

"Professor Hart was born December 25, 1874, at Sandusky, Ohio. He received his B.S. degree from Michigan in 1897 and later attended the Universities of Heidelberg, and Marburg, Germany, in 1900-1901. His first



EDWIN BRET HART

position in this country was with Dr. L. L. Van Slyke of the New York Experiment Station at Geneva, where he was assistant chemist from 1897 to 1902 and associate chemist from 1902 to 1906. He was appointed Professor of Biochemistry and Chairman of the Department of Biochemistry at the University of Wisconsin in 1906, which positions he holds today.

"Among the numerous meritorious contributions to fundamental dairy knowledge which the Committee found in Professor Hart's record, the following are cited as deserving of special mention:

"(1) The determination of phosphorus in feeds and the rôle of phosphorus in nutrition of animals. Also his work on the chemical changes which take place in ripening cheese. This work is the most complete and significant that has ever been done on this subject.

"(2) The relationship of copper and iron for building blood hemoglobin, in the prevention or cure of nutritional anemia.

"(3) The importance of minerals other than iron and copper in animal nutrition, especially phosphorus and its availability from both organic and inorganic sources; iodine in the prevention of goitre, referred to as 'big neck' in ruminants; and magnesium as supplied by dolomitic limestone, which is the prevailing limestone in many parts of the country.

"(4) A fuller understanding of the function of protein in dairy and livestock nutrition, the supplementary relationships of proteins from different plant and animal sources, and the place of simple forms of nitrogen such as urea and ammonium compounds as sources for protein building. Few research men in any country have done more effective work than Professor Hart in the protein nutrition of dairy cattle.

"(5) The existence of the 'grass juice factor' in animal nutrition, which has particular reference to summer milk, and to winter milk which has been produced on superior roughages.

"(6) The favorable effect of fat on the utilization of lactose in milk.

"(7) The superior value of butterfat over vegetable oils, through virtue of certain essential fatty acids.

"Last, but not the least, the Committee wishes to call especial attention to Professor Hart's leadership in training scientists. Many brilliant young men have sought an opportunity to work with Professor Hart in his laboratory at Madison. Many of these Hart-trained men are now leaders in many experiment stations and in industry."

In the absence of the recipient, Mr. Gus Bohstedt of the University of Wisconsin, was called to the platform. Mr. W. A. Wentworth of the Borden Company presented Mr. Bohstedt the gold medal and a check for \$1,000 and requested him to carry it to his colleague, Professor E. B. Hart.

Mr. Wentworth then gave a summary of the awards granted during the past five years and assured the Association of their continuation for at least one more year.



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COWS' URINE AS A FERTILIZER FOR BLUEGRASS PASTURES

W. B. NEVENS

Illinois Agricultural Experiment Station, Urbana, Illinois

Most dairy farmers recognize the fact that dairy cows' urine has some value as fertilizer, but as a rule, its true value is not appreciated and it is not as well conserved and utilized in crop production as the solid portion of the excreta. The object of the investigation reported herewith was to call attention to the high value of cows' urine as a fertilizer by demonstrating its effects upon bluegrass pasture.

The urine of dairy cows normally contains one-third to one-half of the nitrogen and three-fourths or more of the potassium in the excreta (feces and urine) of these animals. As much as 12 to 16 pounds of nitrogen and 10 to 12 pounds of potassium may be found in the urine for every ton of feces excreted (6).

Only a few reports of experimental studies of the value of urine as a fertilizer for pastures are to be found in the literature.

Curtiss (1) reported that the application to bluegrass pasture of 4000 pounds of urine containing .7 per cent nitrogen, .167 per cent potash, and .033 per cent phosphorus, increased the yield of grass 26.5 per cent, or equivalent to 650 pounds of hay per acre.

Ernest (2) states that "Danish experiments with urine have shown that the effect of an autumn application was only 30 to 40 per cent that of a spring application." (Abstract taken from Pieters (8).)

Falke (3) found that the effect of fertilizers on naturally established pastures was to increase "the percentage of protein as well as the digestibility of the protein except that Plot 8 which received urine rather than nitrate of soda fell below the others in production." (Abstract taken from Pieters (8).)

Zacharewicz (10) reported that the use of liquid manure, superphosphate, complete chemical fertilizer, and stable manure, increased the yields of a meadow 17 per cent, 48 per cent, 78 per cent, and 69 per cent, respectively, based upon the yields of check plots.

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EXPERIMENTAL METHODS

Four plots, each 2×2 rods in size, were laid out in a well-sodded and nearly level part of a 4.5-acre bluegrass pasture. Short posts were set at the corners of each plot and the plots were separated from each other by 1-rod borders. No fencing other than the posts was used so that the cattle grazing in the pasture had free access to all of the plots. Reinforced wire cages approximately $4' \times 4'$ in size were used to protect sampling areas within each of the plots (Fig. 1). Cages were also placed in the pasture nearby to facilitate the sampling of a control area.

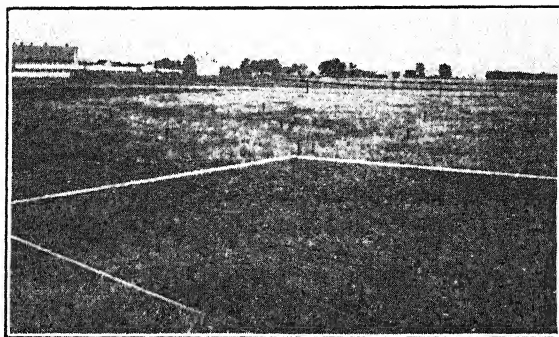


Fig. 1. The fertilized plots (marked by short posts) were grazed closely while the grass on the unfertilized portions of the pasture headed out. Plot 4 (marked by white lines) is in the immediate foreground. Plot 1 in the background shows but little more intensive grazing than the unfertilized pasture. Photographed July 4, 1939.

The first samples of the season were harvested immediately prior to turning the cattle to pasture. Only one area on each plot was harvested on the first sampling date. During the taking of the first samples, two wire cages were placed at each sampling location. One of these was placed over an area just harvested. The forage taken from this area the following month comprised only the forage produced by one month's growth. This was designated the "A" sample.

On each sampling date a cage was placed over a representative portion of the open pasture. The forage harvested from this protected area the following month formed the "B" sample. It comprised not only forage produced during the one month's growth, but also the forage on the area at the time the protecting cage was put in place the previous month. Hence, in computing the yields by the "B" method, the amounts of forage on the open pasture the month previously as determined from the "C" samples were subtracted from the "B" samples.

The "C" samples consisted of harvests of forage from the open, or unprotected, portion of each plot on each harvest date. These samples indicate

only the amounts of forage on the pasture at the time of harvest and used alone do not represent yields.

The samples were harvested by the use of a metal frame and grass shears (7). The metal frame was 44" × 44" in size and 1½" high. It was braced by cross rods. A flat sliding bar laid over the top formed a guide for the shears to insure cutting of the forage at the same height each time.

The forage was collected in cloth sacks and taken to the laboratory where it was at once separated by careful hand sorting into weed and grass portions. Each portion was resacked in tared cloth sacks, weighed, and dried in a constant-temperature electrically-heated oven at 95°–100° C. The grass portion, which consisted almost entirely of Kentucky bluegrass (*Poa pratensis*), was analyzed for its nitrogen content.

The urine was collected from high-producing dairy cows into clean pails during the act of urination. It was applied by hand within a few hours after collection by means of garden sprinkling cans. In most cases it was applied undiluted. Because of a low moisture content in the surface soil, the urine applied May 13, 1939, was mixed with an equal volume of water with the object of preventing injury to the grass.

Applications of urine were made in April, May, and June of 1939, and in May and June of 1940. The rates of application in pounds per acre to Plots 1, 2, 3, 4, and 5 were 1250 pounds, 2500 pounds, 3750 pounds, 5000 pounds, and 0 pounds, respectively. The first treatment in 1940 was delayed until after samples of the forage had been taken, in order to determine if there was a carryover effect of the previous year's treatment. The nitrogen content of the different lots of urine ranged from 1.068 per cent to 1.29 per cent and the potassium content from 0.91 per cent to 1.22 per cent.

EXPERIMENTAL RESULTS

Heavy applications of nitrogenous fertilizers to grasses sometimes cause "burning," an injury which may temporarily retard growth or in some instances completely kill the plants. No burning, or injury, of the grass was noted following the application of urine except after the treatment made June 8, 1940. In spite of apparently ample moisture in the soil from a recent rain, some burning of the grass occurred in the two most heavily fertilized plots, especially on the small areas from which samples had been harvested two days before. The urine applied on that date carried more than 1 per cent nitrogen and approximately the same percentage of potassium, or slightly more than 20 pounds of each of these elements per ton of urine. The applications were such that larger quantities of nitrogen and potassium were applied annually than is customary in the use of commercial fertilizers.

Striking differences were found in the protein content of the grass harvested from the various plots (tables 1 and 2). Several generalizations may be drawn from the data, viz.:

TABLE 1
Composition of samples harvested from bluegrass pasture plots in 1939

Plot No.	Date of harvesting samples									
	May 5		June 7		July 5		Aug. 8		Sept. 11	
	Dry matter content	Protein in dry matter	Dry matter content	Protein in dry matter	Dry matter content	Protein in dry matter	Dry matter content	Protein in dry matter	Dry matter content	Protein in dry matter
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent

“A” Samples

1	36.0	18.4	40.3	12.5	33.3	14.8	35.7	14.4	40.6	14.6
2	36.5	19.2	44.3	14.1	*	*	14.8	15.6
3	32.1	21.2	37.4	18.7	31.7	16.4	26.3	17.4	35.7	16.1
4	32.7	21.6	40.7	16.3	34.7	18.6	36.4	19.1	39.0	16.6
5	30.8	16.7	38.9	10.6	36.5	12.6	33.3	15.0	38.0	16.7

“B” Samples

1	36.0	18.4	41.7	11.0	44.5	10.8	50.0	10.3	43.3	11.3
2	36.5	19.2	47.7	10.8	39.1	12.5	41.0	11.6	39.5	13.9
3	32.1	21.2	39.3	14.8	34.8	14.7	43.1	12.5	41.2	16.8
4	32.7	21.6	43.7	16.5	35.4	18.3	40.4	15.1	39.3	16.9
5	30.8	16.7	44.2	9.5	42.0	10.2	49.4	10.1	41.5	13.8

* Cage moved by cattle; no sample.

TABLE 2
Composition of samples harvested from bluegrass pasture plots in 1940

Plot No.	Date of harvesting samples									
	May 2		June 6		July 5		Aug. 12		Sept. 26	
	Dry matter content	Protein in dry matter	Dry matter content	Protein in dry matter	Dry matter content	Protein in dry matter	Dry matter content	Protein in dry matter	Dry matter content	Protein in dry matter
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent

“A” Samples

1	25.0	17.7	29.8	11.9	31.5	13.4	34.1	12.6	33.3	18.6
2	25.0	27.0	27.5	12.0	26.4	16.1	30.8	12.7	35.3	21.6
3	17.6	19.6	29.1	14.1	29.1	17.5	33.3	13.3	33.3	19.4
4	21.2	18.4	27.8	13.7	31.7	17.6	27.3	12.8	35.7	23.8
5	24.4	16.8	24.1	10.5	37.0	12.1	11.0	37.5	17.4

“B” Samples

1	25.0	17.7	31.1	9.0	36.9	10.2	48.1	8.6	52.0	12.7
2	25.0	27.0	29.4	10.9	37.1	10.9	45.4	9.4	50.0	*
3	17.6	19.6	29.1	11.8	35.5	16.1	48.2	8.8	42.9	17.1
4	21.2	18.4	30.9	11.1	37.1	13.3	45.9	13.4	40.0	17.4
5	24.4	16.8	33.3	8.3	44.7	7.4	36.6	9.4	54.5	13.4

* Sample lost.

(a) In most instances the protein content of the grass on the treated plots was higher than that of the untreated, and the larger the amount of urine applied, the higher the protein content. Exceptions occurred during nearly dormant conditions of the pasture in August and September of both years, and in several instances the protein content of Plot 3 was higher than that of Plot 4.

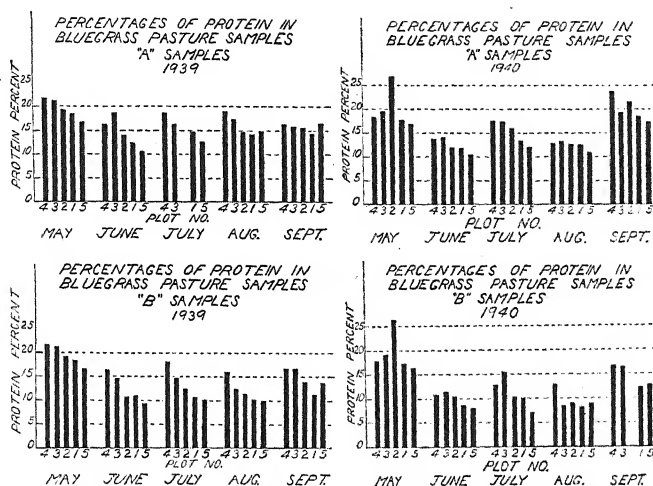


FIG. 2. Treatment of bluegrass pasture plots with cows' urine increased the protein content of the grass over that of the control area (Plot 5), and during the early part of the season, the heavier the application, the higher the protein content of the grass.

(b) The protein content of the A samples was higher than that of the B samples. This is attributed to the fact that the A samples represented only new growth, while the B samples comprised both new growth and older forage. In comparing the analyses of the A and B samples given in tables 1 and 2, it should be noted that the analyses of the samples harvested May 5, 1939, and May 2, 1940, have been listed under both A and B samples in order to facilitate comparison of the A and B samples with the first samples of the season. Following the terminology used in this report, these first samples were neither A nor B samples, but were C samples.

(c) The protein content declined rapidly with advancing development of the plants and dry weather. Rainfall during the summer months of 1940 was less than during the corresponding period of 1939 (table 3) and the protein content of the samples harvested in the summer of 1940 was somewhat less. Light showers during the latter part of August, and in September, 1940, stimulated some new growth with a consequent rise in the protein content of the harvest made Sept. 26, 1940. In this experiment a renewed growth induced by rain seems to have been fully as potent a factor or an even more important factor than fertilization in enhancing the protein content of the grass.

TABLE 3
*Rainfall at Urbana, Illinois**

Month	Average 1889-1940 incl.	1939	1940
	<i>inches</i>	<i>inches</i>	<i>inches</i>
April	3.53	5.39	3.96
May	3.87	1.19	4.53
June	3.73	6.17	5.04
July	3.08	1.73	0.95
August	3.34	6.38	2.80
September	3.22	0.32	0.48
October	2.43	2.54	1.93
Total for year	35.03	38.05	30.60

* University of Illinois Cooperative Weather Bureau.

(d) There was a carryover effect of the urine treatments of 1939 which lasted not only throughout the sampling period but was also evident in the samples harvested May 2, 1940. The unusually high protein content found for Plot 2 on May 2, 1940, is unexplained even after a repetition of the analysis. However, even after leaving out of consideration this unusually high figure, the protein content of the grass from the other three plots was found to be substantially higher than that of Plot 5, the control area. As pointed out above, the first application of urine in 1940 was not made until after these samples had been harvested.

TABLE 4
Yields of dry matter and amounts of dry matter in open pasture on bluegrass pasture plots in 1939

Method of determination	Amounts per acre of dry matter				
	Plot No.				
	1	2	3	4	5
	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>
"A" grass	3143	3095	3435	4310	2317
"A" weeds	161	38	308	292	259
Total	3304	3133	3743	4602	2576
"B" grass	4083	3110	3857	3984	2771
"B" weeds	162	26	614	16	49
Total	4245	3136	4471	4000	2820
"C" grass	3726	2511	2170	1685	4519
"C" weeds	16	6	114	97	32
Total	3742	2517	2284	1782	4551

The yields of dry matter in the forage are shown in tables 4 and 5. The yields as determined by the A method, or the harvests of the new growth (tables 4 and 5), were larger for the treated plots (Nos. 1-4) than for the control plot (No. 5). Also, the June and July harvests of 1939 indicated that the larger the application of urine the larger the yield of dry matter in

the bluegrass. Low rainfall in July and also during the latter part of the pasture season of both years was followed by such low yields that no direct relation between method of treatment and yield was apparent.

TABLE 5

Yields of dry matter and amounts of dry matter in open pasture on bluegrass pasture plots in 1940

Method of determination	Amounts per acre of dry matter				
	Plot No.				
	1	2	3	4	5
	lbs.	lbs.	lbs.	lbs.	lbs.
"A" grass	3402	3694	2980	3759	2041
"A" weeds	65	97	567	486	97
Total	3467	3791	3547	4245	2138
"B" grass	3013	4180	4018	2268	1103
"B" weeds	0	97	728	339	470
Total	3013	4277	4746	2607	1573
"C" grass	4309	3564	1684	3531	3790
"C" weeds	97	65	113	292	48
Total	4406	3629	1797	3823	3838

The yields as determined by the B method (*i.e.*, B samples—C samples of previous month) are for the most part in substantial agreement with those determined by the A method, particularly with respect to the higher yields of the treated plots than of the untreated. Considering the small size of the areas harvested in sampling, the agreement of the two methods seems remarkably good. The merits of these methods of determining yields have been discussed by Fuelleman and Burlison of this Station (4, 5).

Plots 1-4 were nearly bare from July to September, 1939, and during this period the amounts per acre of forage on these plots were less than on Plot 5, the control plot. Probably this closely grazed condition of the treated plots in the fall of 1939 accounted for smaller yields from them than from Plot 5 in May, 1940. A summary of the yields for 1940 is given in table 5. In spite of low yields of Plots 1-4 in May, 1940, the yields of these plots for the season, as determined by both the A and the B methods, were much higher than for the check plot.

The palatability of the grass on the urine-treated plots was apparently greater than that of the untreated portions of the pasture. Within a few weeks after the cattle were first turned to pasture in 1939, it was evident that Plot 4 and a little later Plot 3 were being grazed more heavily than the other plots, or the untreated pasture. A short time after, Plot 2, and finally Plot 1, were given more attention by the cattle. On the treated plots the grass was grazed closely except around droppings, while on the borders between the plots and the rest of the pasture the grass headed out (Fig. 1). It appears from the data that there was a direct relationship between the

protein content and the palatability of the bluegrass, *i.e.*, the higher the protein content, the greater the palatability.

SUMMARY AND CONCLUSIONS

Four small plots of Kentucky bluegrass were treated in April, May, and June of 1939, and again in May and June of 1940, with applications of cows' urine at rates ranging from 1250 pounds to 5000 pounds per acre at each application. A control area was untreated. Samples of the grass were harvested monthly from May to September, inclusive.

Although the urine contained more than 1 per cent nitrogen and in most cases more than 1 per cent potassium, the heavy applications were, as a rule, not harmful to the forage.

The protein content of the grass on the treated plots was higher than that of the grass on the control area, and in most instances, the heavier the application of urine, the higher the protein content.

The protein content of the A samples, representing recent growth, was higher than that of the B samples, which included both older forage and recent growth. Advancing development of the plants and renewed growth induced by rains were important factors affecting the protein content of the grass, the former causing a decline and the latter an increase in protein content.

The effect of the first year's spring treatment with urine on the protein content of the grass was evident during the remainder of the pasture season and also in May of the following year.

The yields of the urine-treated plots were higher than that of the untreated pasture and there was a tendency toward higher yields from the more heavily treated plots.

The palatability of the grass, as evidenced by close grazing by cattle, was higher on the urine-treated plots than on the untreated pasture and the greater the protein content of the grass, the greater the intensity of grazing.

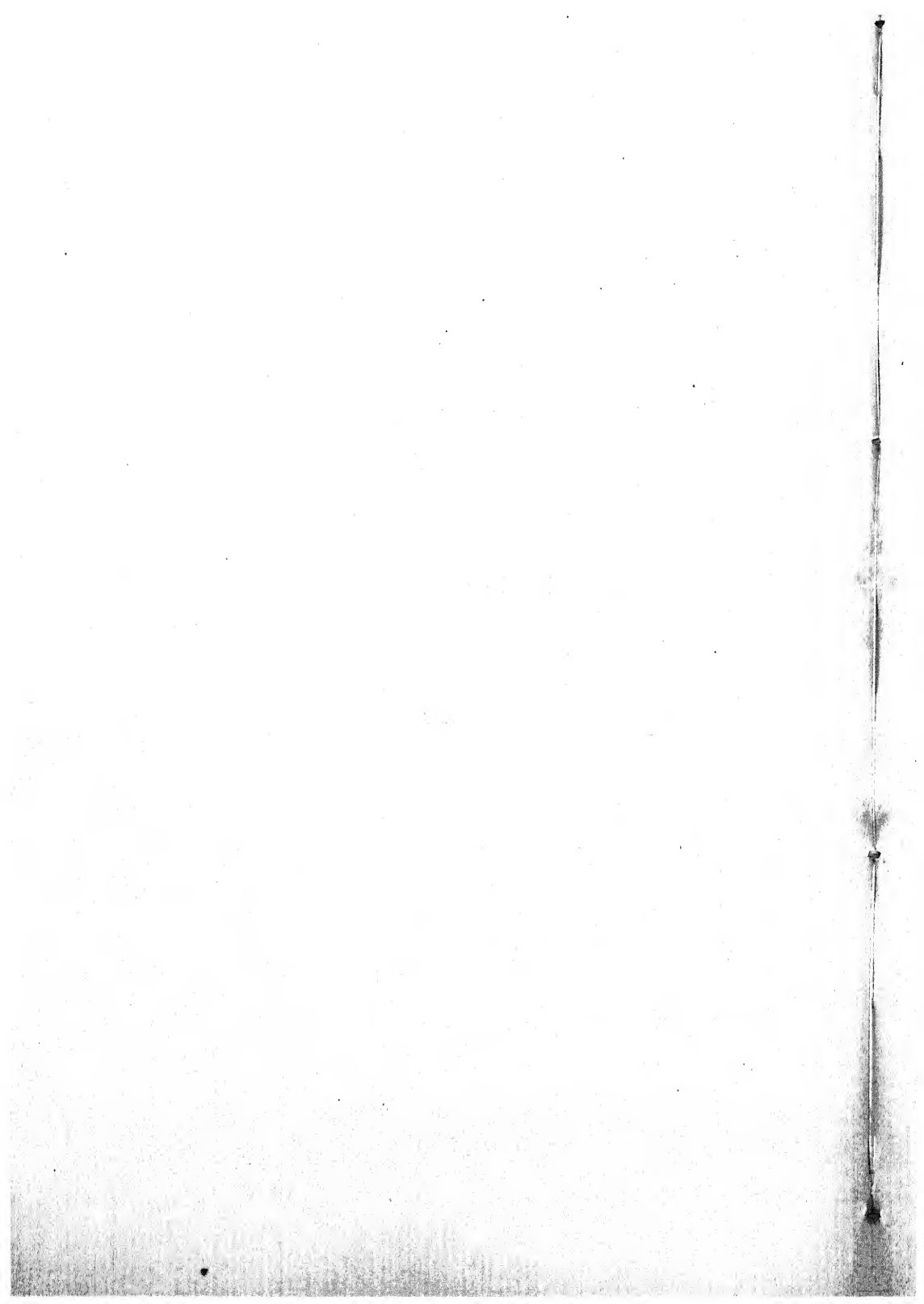
ACKNOWLEDGMENT

This paper constitutes a report of an investigation of pasture improvement methods conducted cooperatively by the Departments of Agronomy, Animal Husbandry, and Dairy Husbandry, University of Illinois. The assistance and counsel of members of these Departments is hereby gratefully acknowledged. The chemical analyses reported were made under the direction of Mr. J. M. Lindgren of the Applied Chemistry Testing Laboratory of the University of Illinois.

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RANCIDITY STUDIES ON MIXTURES OF RAW AND PASTEURIZED HOMOGENIZED MILK*

P. B. LARSEN, G. M. TROUT AND I. A. GOULD

Department of Dairying, Michigan State College, East Lansing, Michigan

When raw milk is homogenized there is an immediate and continued rise in the titratable acidity (1, 4), accompanied by the development of a rancid flavor. Pasteurization of the milk prevents rancidity from developing. The phenomenal development of rancidity in homogenized raw milk has been attributed to the action of lipase normally present in all milk, through activation of the lipase itself, through the creation of new surfaces more susceptible to lipase action, or through the increased surface area of the fat globules. This lipolytic response to the homogenization of raw milk is recognized by the market milk industry to the extent that pasteurization is a closely allied process to the homogenization of milk for bottling purposes.

Even though pasteurized milk will not develop rancidity upon homogenization, nevertheless, information is available indicating that rancidity may be induced in this pasteurized milk by the addition of raw homogenized milk. For example, Dorner and Widmer (2) stated that "mixtures of pasteurized and homogenized cream or milk with raw milk, raw skim milk, or raw cream become rancid." Gould and Trout (3) have demonstrated that in mixtures of homogenized raw and unhomogenized pasteurized milk lipolysis proceeded to a greater extent than if the fat splitting had been calculated as having occurred only in the raw product.

In the study herein presented combinations of homogenized and unhomogenized raw and homogenized and unhomogenized pasteurized milk were made to ascertain under what conditions and to what extent rancidity would occur.

EXPERIMENTAL PROCEDURE

Raw milk was secured from the College milk supply which was composed largely of mixed milk from herds of several producers as well as that from the College herd. Lots of the milk were prepared which consisted of unhomogenized raw, homogenized raw, unhomogenized pasteurized and homogenized pasteurized milk. Homogenization was at 2500 pounds pressure, with the milk at approximately 100° F. in the case of the raw milk and at the pasteurization temperature in the case of the pasteurized milk. Pasteurization was conducted at 145° F. for thirty minutes.

The following mixtures of milk were prepared: (a) unhomogenized raw milk with homogenized pasteurized milk at a rate so that the samples con-

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tained 0, 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, 99, and 100 per cent of raw milk; (b) homogenized raw milk with homogenized pasteurized milk in the same proportions as stated above; and (c) unhomogenized raw milk with homogenized raw milk in the same ratios as in (a) and (b). These samples were titrated for increases in acidity, conveniently expressed as lactic acid, with 0.05N NaOH, and were studied organoleptically for the development of rancid flavor immediately after processing and preparing the various mixtures and after 1, 3, 7 and 10 days of storage at 35° to 40° F. The increase in titratable acidity was determined by subtracting the acidity of the unprocessed raw milk after the various storage periods from the titratable acidity of the mixtures after similar storage. The degree of rancidity was expressed numerically as follows: 0, no rancidity; 1, questionable; 2, slightly rancid; 3, distinctly rancid; 4, pronounced rancid. The numerically averaged flavor scores represent the average scores of two or more judges.

EXPERIMENTAL RESULTS

Lipolytic activity in mixtures of unhomogenized raw and homogenized pasteurized milk. The acidity data obtained from this series are portrayed in figure 1. The flavor results are shown in table 1.

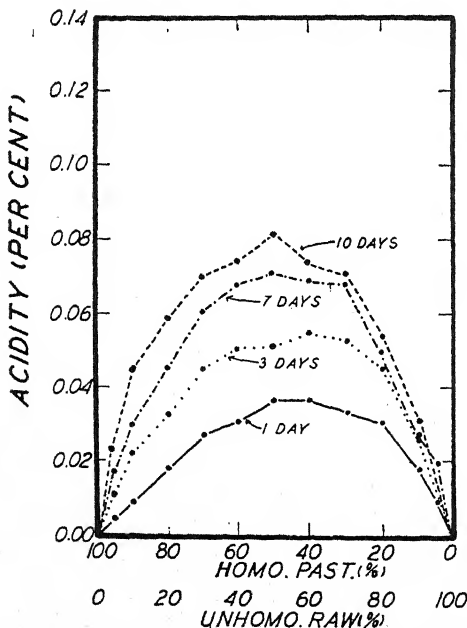


FIG. 1. The increase in acidity after different storage periods when unhomogenized raw milk was added to homogenized pasteurized milk in different proportions.

After three to five days storage, a slight increase in acidity over the control lot was noted in the homogenized pasteurized milk which contained as

little as one per cent of unhomogenized raw milk. When 5 per cent of unhomogenized-raw milk was added to the homogenized pasteurized milk an increase in acidity was observed after one day of storage. As the percentage of unhomogenized raw milk in the homogenized pasteurized milk was increased up to 50 per cent, a progressive increase in the titratable acidity occurred. The maximum increase in acidity was encountered when the ratio of unhomogenized raw milk to homogenized pasteurized milk was approximately one to one. Beyond this point, increased increments of unhomogenized raw milk resulted in a progressive decrease in acidity from the maximum. Small quantities of homogenized pasteurized milk in unhomogenized raw milk, such as one and three per cent, were sufficient to produce an increase in acidity after one to three days of storage, but seemed only slightly more effective in producing an increase in acidity than similar quantities of unhomogenized raw milk in the homogenized pasteurized milk.

TABLE 1

Development of rancidity due to lipolysis in milk made by mixing unhomogenized raw milk with homogenized pasteurized milk. (Average of three trials)

Sample		Rancidity* after			
% Unhomo. raw	% Homo. pasteurized	1 day	3 days	7 days	10 days
0	100	0.00	0.00	0.00	0.00
1	99	0.00	0.00	0.33	0.67
5	95	0.00	0.33	1.00	2.67
10	90	0.33	1.00	2.33	2.67
20	80	2.00	2.67	3.33	3.67
30	70	2.00	3.00	3.33	3.67
40	60	2.33	4.00	4.00	4.00
50	50	2.67	4.00	4.00	4.00
60	40	2.67	4.00	4.00	4.00
70	30	2.33	4.00	4.00	4.00
80	20	2.67	3.00	3.33	3.67
90	10	2.00	2.00	3.00	3.33
95	5	0.67	0.33	1.33	3.00
99	1	0.00	0.00	0.33	0.33
100	0	0.00	0.00	0.00	0.00

* Flavor intensity designated by numerical values ranging from 0 (no rancidity) to 4 (pronounced rancidity).

A questionable rancid flavor was detected in some samples of homogenized pasteurized milk containing one per cent of unhomogenized raw milk after 7 to 10 days of storage, whereas the flavor was pronounced in those samples which contained five per cent of unhomogenized raw milk after the same storage period. A further increase in the percentage of unhomogenized raw milk added to the homogenized pasteurized milk caused a more intense rancid flavor to develop and also produced the flavor more rapidly. All the samples containing from 10 to 90 per cent of unhomogenized raw milk developed a

pronounced rancid flavor upon storage, with the flavor being definite after one day of storage in mixtures of 20 per cent or more. When the sample contained less than 10 per cent of homogenized pasteurized milk in un-homogenized raw milk the speed and intensity of the rancid flavor development was decreased.

Lipolytic activity in mixture of homogenized raw and homogenized pasteurized milk. The acidity data obtained from mixtures of homogenized raw and homogenized pasteurized milk are presented graphically in figure 2

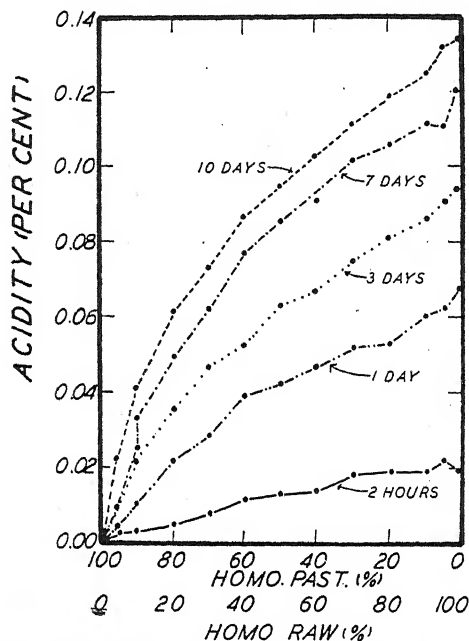


FIG. 2. The increase in acidity after different storage periods when homogenized raw milk was added to homogenized pasteurized milk in different proportions.

and the flavor results are presented in table 2.

When the homogenized raw milk was added to homogenized pasteurized milk, the acidity developed progressively as the increments of homogenized raw milk were increased. In the previous experiment the maximum acidity development occurred when the unhomogenized raw milk and homogenized pasteurized milk were mixed at a ratio of 1:1. In this experiment the maximum acidity developed in the 100 per cent homogenized raw milk. The slightly accelerated and persistent rate of lipolysis with such mixtures might be expected inasmuch as the maximum fat globule surface areas produced by the condition of the experiment were present throughout the series since both lots were homogenized. The development of rancid flavors, in general, closely followed the changes in titratable acidity.

TABLE 2

Development of rancidity due to lipolysis in milk made by mixing homogenized raw milk with homogenized pasteurized milk. (Average of three trials)

Sample		Rancidity after			
% Homo. raw	% Homo. pasteurized	1 day	3 days	7 days	10 days
0	100	0.00	0.00	0.00	0.00
1	99	0.00	0.00	0.00	0.67
5	95	0.00	1.33	1.66	2.33
10	90	0.00	1.66	3.00	3.33
20	80	1.33	2.67	3.67	4.00
30	70	1.33	3.00	4.00	4.00
40	60	2.00	3.67	4.00	4.00
50	50	2.67	3.67	4.00	4.00
60	40	3.33	3.67	4.00	4.00
70	30	3.33	3.67	4.00	4.00
80	20	3.67	4.00	4.00	4.00
90	10	3.67	4.00	4.00	4.00
95	5	3.67	4.00	4.00	4.00
99	1	4.00	4.00	4.00	4.00
100	0	4.00	4.00	4.00	4.00

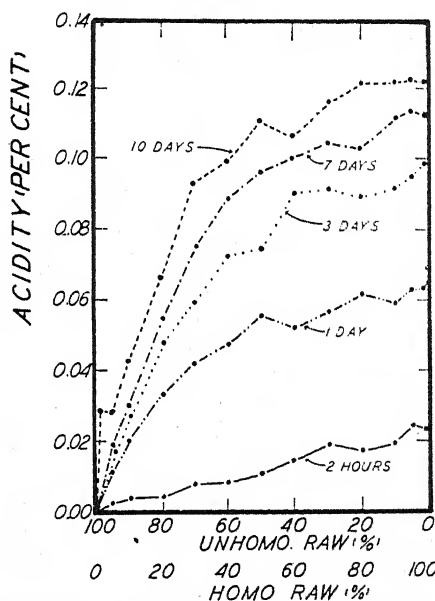


FIG. 3. The increases in acidity after different storage periods when homogenized raw milk was added to raw milk in different proportions.

Lipolytic activity in mixtures of unhomogenized and homogenized raw milk. The results secured when unhomogenized and homogenized raw milk were mixed in various proportions are shown by figure 3 and in table 3.

The same general trend in the development of rancidity and increased

acidity was noted in this series as with the second series. There seemed to be one exception, however; the acidity developed at a slightly faster rate as the percentage of homogenized raw milk in the unhomogenized raw milk increased up to 50 per cent, beyond which the increase was neither so rapid nor so great.

TABLE 3

Development of rancidity due to lipolysis in milk made by mixing raw milk with homogenized raw milk. (Average of three trials)

Sample		Rancidity after			
% Homo. raw	% Homo. pasteurized	1 day	3 days	7 days	10 days
0	100	0.00	0.00	0.00	0.00
1	99	0.00	0.00	0.00	0.00
5	95	0.00	1.00	1.00	2.00
10	90	1.50	2.00	2.00	2.50
20	80	2.00	2.50	3.00	3.50
30	70	2.00	3.50	3.50	3.50
40	60	2.00	4.00	4.00	4.00
50	50	2.00	4.00	4.00	4.00
60	40	2.00	4.00	4.00	4.00
70	30	4.00	4.00	4.00	4.00
80	20	4.00	4.00	4.00	4.00
90	10	4.00	4.00	4.00	4.00
95	5	4.00	4.00	4.00	4.00
99	1	4.00	4.00	4.00	4.00
100	0	4.00	4.00	4.00	4.00

Likewise, a slightly more intense rancid flavor was noted at one and three days in mixtures of homogenized raw with raw milk than similar mixtures with homogenized pasteurized milk. However, at the 10-day storage period, little difference was noted in the intensities of rancid flavor at comparable concentrations of homogenized raw milk.

DISCUSSION AND SUMMARY

A rancid flavor and an increase in acidity were found to develop readily on storage when raw milk was mixed with homogenized pasteurized milk. The results secured confirm earlier work (2, 3), that lipolytic activity is not confined solely to homogenized raw milk but to homogenized pasteurized milk as well provided active lipase is present. The maximum increase in acidity occurred when the ratio of raw milk to homogenized pasteurized milk was approximately one to one. As the percentage of raw milk in the homogenized pasteurized milk increased above 50 per cent, the increase in titratable acidity was found to be correspondingly less. When only a small percentage of the sample was homogenized pasteurized milk, very small increases in acidity occurred. These increases in titratable acidity were closely associated with the development of a rancid flavor.

The fact that the greatest increases in acidity in mixtures involving pasteurized milk occurred when the milk was approximately 50 per cent unhomogenized raw and 50 per cent homogenized pasteurized indicates that the amount of increased surface or increased surface activation caused by homogenization and the amount of lipase added by the raw milk are of approximately equal importance in the development of rancidity in homogenized milk. It would appear, therefore, that increases in acidity and the development of rancidity in homogenized raw milk are dependent upon the factors concerned with the increased surface and not upon an activation of lipase by homogenization.

Further evidence of the equal importance of the fat surfaces and the amount of lipase present is shown by the fact that when homogenized raw milk was added to homogenized pasteurized milk the rate of increase in acidity was only slightly greater than when unhomogenized raw milk was mixed with homogenized pasteurized milk. If lipase is activated by homogenization it would seem that these increases should have been considerably faster than those noted. The greater increase which did occur in the homogenized raw and homogenized pasteurized milk mixtures might be explained by the fact that all of the fat had been subjected to homogenization so that there was more fat surface exposed upon which the lipase could act than in the raw milk and homogenized pasteurized milk mixtures in which only a portion of the fat had been subjected to homogenization. In the latter case the amount of lipase added by the raw milk seemed to be the limiting factor in the development of rancidity. The lipase added to the homogenized pasteurized milk in the form of unhomogenized raw milk appeared to be just as effective in causing rancidity as was the lipase added by the homogenized raw milk.

From these studies it would seem that homogenized pasteurized milk contaminated with raw milk is as susceptible to lipolysis as homogenized raw milk. In addition, these results indicate the possibility of controlling the extent of the development of rancidity through the use of proper mixtures of homogenized pasteurized and raw milk.

CONCLUSIONS

Rancidity developed readily in mixtures of milk composed of (a) unhomogenized raw milk and homogenized pasteurized milk, (b) homogenized raw milk and homogenized pasteurized milk, and (c) unhomogenized raw milk and homogenized raw milk.

The development of rancidity seemed to be equally dependent upon the amount of lipase present and upon the amount of acceleration afforded by the newly created surfaces.

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EFFECT OF CERTAIN FACTORS UPON LIPOLYSIS IN HOMOGENIZED RAW MILK AND CREAM*

I. A. GOULD

Department of Dairying, Michigan State College, East Lansing, Michigan

The acceleration of lipase action in raw milk by homogenization is now generally accepted. This acceleration has been attributed by some to increased surface area afforded the lipase by the breakdown of the fat globules and by others to a re-surfacing of the fat globules by material more susceptible to lipolytic action. Irrespective of the actual cause for the enormous and rapid rate of lipolysis in homogenized milk, evidence is accumulating which indicates that factors which affect lipase action in normal milk may not have the same effect on lipase activity in the homogenized product. A limited amount of information illustrating these differences has already been published. Additional evidence is presented in this paper.

Lipolytic action on fat in homogenized milk has been previously studied (3, 4, 7). Gould and Trout (4) found the acid degree of the fat (expressed in milliliters of N/1 NaOH per 100 grams of fat) to increase four-fold to six-fold within a few minutes after homogenization, and to increase on an average of 1,652 per cent within 24 hours. The author (3) observed considerable lipolysis to have occurred in fat obtained from milk homogenized at temperatures of 105 to 135° F., whereas slight fat splitting occurred in milk homogenized at 145° F. These temperatures are considerably above those which have been found to be effective in greatly inhibiting lipase action in normal unhomogenized milk (9, 10, 11, 12).

Lipase action in homogenized milk is apparently not affected by temperature activation which brings about marked changes in normal milk (6, 7). Krukovsky and Sharp (7) believe the difference is due to the fact that in the homogenized product the "lipase is already in the active state as a result of the resurfacing of the milk fat. . . ."

Another point of difference between lipase activity of normal and homogenized milk pertains to the temperature coefficient. Krukovsky and Sharp (7) found the temperature coefficient of the lipase action to differ depending upon whether the fat globules were normal or whether they had been "resurfaced." Fat globules with natural surfaces showed more rapid lipolysis with lower temperature whereas a reverse condition occurred with the emulsified fat.

A relationship between oxidative changes in the fat and lipolysis in normal milk is indicated by Davies (1) and Krukovsky and Sharp (8). Davies found peroxide formation to occur simultaneously with lipase action; the

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peroxides being formed from oxidation of oleic acid which was freed by the fat splitting enzyme. This worker also found copper to be effective in inhibiting lipase activity, with 2 p.p.m. reducing lipolysis by approximately 70 per cent. Herrington and Krukovsky (5) found lipase action was reduced about 20 per cent by 0.2 and 0.4 p.p.m. of copper. Later, Krukovsky and Sharp (8) showed that the inactivating effect of copper, even in amounts of 2 to 8 p.p.m., was almost entirely prevented by removing the dissolved oxygen in the milk. They further found that the removal of oxygen increased the resistance of normal milk lipase to inactivation by heat. In earlier work, Dorner and Widmer (2) were unable to prevent rancidity in homogenized milk by removal of oxygen. However, rancidity was prevented by addition of carbon dioxide, but this was thought to be due to increases in the acidity of the milk by the gas.

In the study herein reported, results are presented which deal with the lipolysis which occurs in homogenized raw milk or cream and with the influence of certain factors upon the rate and extent of the lipolytic action.

EXPERIMENTAL PROCEDURE

Milk used in these trials was mixed-herd milk secured from the College creamery. Homogenization was at 500–1000 pounds pressure by means of a stainless-steel, commercial-size viscolizer. Milk or cream was homogenized at approximately 100° F., and a similar temperature was used when the raw milk was separated. To stop lipolysis following processing and storage, the milk or cream was pasteurized at 148–150° F. for 30 minutes. When a storage period was involved, a temperature of approximately 40° F. was used unless otherwise specified.

All measurements to determine lipolysis were conducted on the fat. Fat for analysis was obtained by churning the cream, followed by centrifuging and filtering the melted butter oil. The free fatty acids were measured by direct titration with 0.1 N NaOH using the procedure described previously (4). The values are expressed as acid degrees (the number of milliliters of 1.0 N NaOH per 100 grams of fat).

Although churning in many cases was comparatively difficult due both to homogenization and also to the subsequent lipolysis which frequently occurred, nevertheless, it was always possible to secure sufficient fat for the determinations. Perhaps the greatest churning difficulty was encountered with those samples containing relatively large quantities of formalin, this effect doubtless being partly due to the action of formalin on the proteins.

Peroxide values were determined by the Wheeler method (13), and the results are expressed as peroxide number (the millimols of peroxide oxygen in combination with one kilogram of fat).

EXPERIMENTAL RESULTS

Influence of copper on lipolysis. In this experiment, copper was added as a solution of copper sulfate to make concentrations in the milk of 2, 6 and 10 p.p.m. The copper was added to the milk before homogenization in certain trials and following homogenization in others. The results of several trials are shown in table 1.

TABLE 1
*Lipolysis in fat from homogenized milk as influenced by added copper**

Sample	Hours	Trial No.				Ave.
		1	2	3	4	
Control	0	3.67	4.55	3.40	4.20	3.96
	24	11.70	18.10	14.55	14.30	14.66
	72	14.30	23.70	15.20	19.60	18.20
2 p.p.m. Cu	24	12.15	18.80	15.95	13.20	15.03
	72	14.40	25.50	17.30	16.80	18.50
6 p.p.m. Cu	24	11.75	16.95	13.15	11.15	13.25
	72	15.00	24.60	15.20	14.50	17.20
10 p.p.m. Cu	24	11.75	18.80	12.60	13.25	14.10
	72	14.70	24.60	14.50	16.10	17.48

* Values expressed as acid degrees. Copper added following homogenization in first two trials and before homogenization in last two trials.

These results show the copper to have no significant effect on the extent of lipolysis whether added prior to, or subsequent to, homogenization. The acidity values for the copper-containing samples were practically the same as the control samples in every trial after 24 and 72 hours. Average values of the four trials show no distinct trend in fat acidity to accompanying increases in the copper content. The failure of copper, even in comparatively large amounts, to inhibit lipolysis in homogenized milk is at variance with the results reported by others for lipase action in the unprocessed product.

Influence of sodium chloride on lipolysis. Information is lacking concerning the influence of NaCl on lipolysis in homogenized milk or cream, although Pfeffer, *et al.* (10) report that NaCl was found to inhibit lipolysis in the unhomogenized product. Because of the scarcity of information on this subject, trials were conducted in which different concentrations of NaCl were added to homogenized raw cream. In these trials, NaCl was added to the cream, at the rate of 0, 2, 5, and 8 per cent and the cream stored for 72 hours. The results are illustrated by figure 1.

This figure shows NaCl to have an inhibiting effect upon fat splitting, with the effect increasing directly with the salt concentration. The broken line represents the acid degree of the fat at the time of adding the salt. The results show that both the 5 and the 8 per cent levels were sufficient to inhibit lipolysis practically completely. If the 8 per cent concentration is taken

to be 100 per cent efficient in preventing lipase activity, then the calculated efficiencies of the 2 and 5 per cent levels would be 41 and 94 per cent respectively. On the basis of these findings it would appear that the lipase activity in homogenized milk or cream is retarded and even prevented by NaCl.

Influence of formalin on lipolysis. The recent work of Herrington and Krukovsky (5) dealing with the use of formalin in unhomogenized normal milk indicates that even small quantities of this chemical reduced the lipase action to a small fraction of its original value and that larger amounts were

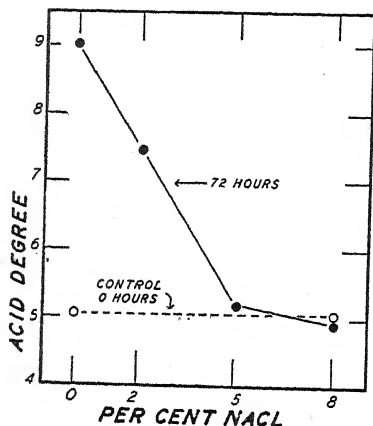


Fig. 1. The influence of sodium chloride on lipolysis in homogenized raw cream.

no more effective. Since the influence of formalin on lipolysis in homogenized milk or cream has not been studied, an experiment was conducted in this connection. Milk was warmed to 100° F., homogenized, and separated. The cream was standardized to 15 per cent fat and divided into 6 lots. These lots were treated as follows: Lot 1—Control—stored 0 hour; Lot 2—Control—stored 72 hours; Lot 3—5 ml. formalin per 3 pounds cream, stored 0 hour; Lot 4—1 ml. formalin per 3 pounds cream, stored 72 hours; Lot 5—3 ml. formalin per 3 pounds cream, stored 72 hours; Lot 6—5 ml. formalin per 3 pounds cream, stored 72 hours. The ratios of the formalin to cream were about 1:1350, 1:450, and 1:250. The results are presented in table 2.

These results show formalin in the amounts used to have no inhibitive effect upon the fat splitting action. The average values show the control lot to have changed from an acid degree of 4.97 to 8.25 within the 72 hour period, whereas the lot containing 1 ml. formalin underwent approximately the same extent of change and those with 3 and 5 mls. of formalin averaged even greater fat splitting during storage. A higher degree of lipolysis in the samples containing 3 and 5 mls. of formalin resulted in two of the three trials conducted.

TABLE 2

*Lipolysis in fat from homogenized milk as influenced by formalin**

Trial No.	Storage period (hours)					
	0		72			
	Formalin (ml.)		Formalin (ml.)			
	0	5	0	1	3	5
1	5.15	4.85	8.25	7.95	11.25	11.40
2	4.05	4.75	8.00	7.75	8.30	7.65
3	5.70	5.60	8.50	8.50	10.80	10.15
Avg.	4.97	5.07	8.25	8.07	10.12	9.73

* Formalin concentration as milliliters per 3 pounds of cream. Values expressed as acid degrees.

Influence of storage temperature on lipolysis. Dorner and Widmer (2), by using direct titration methods on homogenized milk, came to the conclusion that lipolytic activity varied directly with the storage temperature. Since the direct titration on the milk is a less sensitive method of measuring the acidity as produced by lipase activity, it appeared desirable to study the influence of storage temperature by means of fat titration. Therefore, an experiment was conducted in which 20 per cent cream was homogenized and then divided into four lots. Lot 1 was pasteurized at once; Lot 2 was stored for 72 hours at approximately 0° F., Lot 3 was stored for 72 hours at 35° F., and Lot 4 was stored for 72 hours at approximately 70° F. All of the samples were treated with a small amount of formalin (2 ml. per gallon), immediately following pasteurization to prevent excessive bacterial changes during storage. The results are illustrated by figure 2.

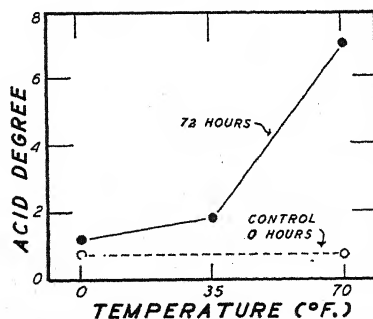


FIG. 2. The influence of the storage temperatures on lipolysis in homogenized raw cream.

These results show the lipase activity in homogenized cream to vary directly with the storage temperature. However, the average increases in free fatty acids at the two lower temperatures were slight, amounting to approximately 0.5 and 1.15 acid degrees for the 0° F. and the 35° F. tem-

peratures, respectively. Much greater lipase activity occurred at 70° F., with the acid degree increasing approximately 6.25 during the 72-hour period. Thus, the lipolytic activity was practically doubled between 0° F. and 35° F., and had increased approximately 12 fold at 70° F. These results had been secured prior to the appearance of the paper by Krukovsky and Sharp (7) dealing with "resurfaced" fat globules, but the same conclusions may be drawn even though the results were secured by somewhat different means, *i.e.*, that lipolysis in homogenized milk displays a normal temperature coefficient.

Influence of pasteurization of different milk fractions on lipolysis. Dorner and Widmer (2) found that when heated homogenized milk was mixed with raw skim milk the product became rancid. They concluded therefore that the causative agent was in the milk serum. Pfeffer, Weckel and Jackson (10) report similar conclusions for unhomogenized milk. In both of these studies the workers were actually referring to milk plasma rather than to milk serum. To study the problem of homogenized milk from the standpoint of changes in the acidity of the fat, trials were conducted in which fractions of 40 per cent cream and skim milk were remixed to make milk testing approximately 6 per cent. This prepared milk was then homogenized. In one lot the cream was pasteurized prior to mixing with the skim milk, in another lot the skim milk was pasteurized, whereas a third lot (the control) consisted of a mixture of the raw cream and raw skim milk. Fat acidity determinations were made at 0, 24, and 72 hours. The results are shown by figure 3.

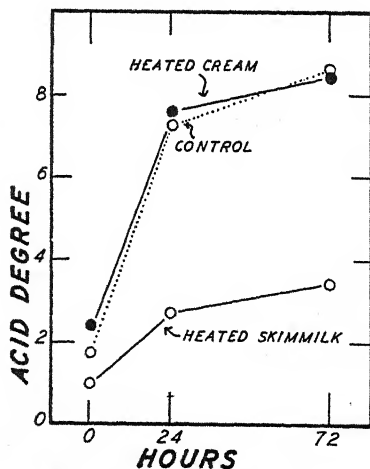


FIG. 3. The influence of heating cream and skimmilk fractions on lipolysis accelerated by homogenization.

This figure shows that although the heating of the skim milk markedly reduced the extent of lipolysis, a similar treatment of the cream had but

slight influence. It may be observed that the rate and amount of lipolytic action was not greatly different between the control lot and the lot in which the cream portion was heated. The fat acidity in the lot in which the cream was heated increased approximately 3.5 fold and the fat acidity in the control samples increased approximately 4.9 fold, thus indicating a somewhat greater increase in lipolysis in the latter. However, the actual percentage increase on the basis of the value at the 0-hour period may be of only secondary importance. For example, the change in fat acidity in the lot containing the heated skim milk amounted to approximately a 3.3 fold increase. Since the value at the 0-hour period was comparatively low, being approximately one-half that of the other lots at the same period, the total increase was much smaller than in the other lots. The increase during the 72 hour period in acid degrees amounted to 2.43 for the heated skim milk lot, as contrasted with 6.1 and 6.85 for the heated cream and control lots, respectively. The lipolysis which resulted when the skim milk fraction was pasteurized is doubtless due to the lipolytic activity of the plasma portion of the raw cream.

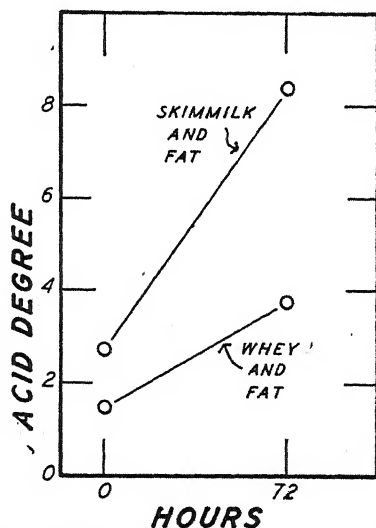


Fig. 4. Differences in lipolysis when fat is dispersed in skim milk and whey.

Influence of fractions of milk plasma on lipolysis. In this experiment, efforts were expended to determine the difference between skim milk and whey in affecting "homogenization" lipolysis. Raw skim milk was divided into two lots. In one lot the casein was precipitated by rennin and the whey filtered free of the coagulated protein. Melted butter oil secured from pasteurized cream was added both to the skim milk and the whey fractions to make a product testing approximately 4 per cent fat. The two lots were then homogenized, and fat was obtained for titration at 0 and 72 hours of storage. The results are portrayed by figure 4.

This figure shows greater lipolysis to occur in the case of skim milk-fat combination, although the whey-fat lot exhibited considerable lipolytic activity. The acid degree of the fat in the whey lot increased from 1.49 to 3.87, an increase of approximately 2.6 fold, whereas the acid degree change in the fat of the skim milk lot was from 2.69 to 8.44, an increase of about 3.1 fold. The fact that the whey was found to exhibit considerable lipolytic activity is at variance with the suggestion of Dorner and Widmer (2) to the effect that whey probably does not contain the agent which caused homogenized raw milk to become rancid. The results of this study do indicate, however, that a considerable portion of the lipolytic activity of milk is removed with the casein.

Relationship of lipolysis to oxidative changes in the fat. The results of Davies (1) and Krukovsky and Sharp (8) with lipase in normal milk indicate that oxidative changes are also involved with the lipolytic action. However, these workers found normal milk lipase to be inhibited by copper, whereas the results presented earlier in this paper show copper to be ineffective in preventing lipolysis in homogenized milk. This indicates that there may be no relationship between oxidative changes and lipolysis in the case of homogenized raw milk.

To determine if oxidative changes were occurring simultaneously with lipolysis, peroxide determinations* were conducted on the fat in the majority of the trials which were made in connection with this study. A summary of the results is given in table 3.

TABLE 3
Peroxide numbers of fat which has undergone different degrees of lipolysis

Number of samples	Acid degrees	Peroxide numbers	
		Average	Range
19	4 or less	0.32	0.00-0.65
4	4.1-6.0	0.31	0.14-0.50
19	6.1-8.0	0.39	0.01-1.00
5	8.1-10.0	0.32	0.15-0.60
35	10.1 or more	0.24	0.04-0.85

The results in this table offer evidence that lipolytic activity, accelerated by homogenization, is not related to oxidative changes, at least under the conditions of this experiment. The peroxide values were not significantly altered by marked changes in the degree of fat splitting and all of the values were relatively low indicating no appreciable amount of fat oxidation. On the basis of these results, it would appear that lipolysis in homogenized raw milk proceeds independently of oxidative changes in the fat.

* Credit is due Mr. R. C. Townley, graduate assistant in Dairy Manufactures, for the peroxide determinations.

DISCUSSION AND SUMMARY

The results secured in this study show that lipase action which occurs in homogenized raw milk usually reacts to external factors differently than does the lipase in normal milk. Other workers (1, 5, 8) have shown normal lipase activity to be greatly inhibited by copper, whereas in the studies herein presented, no such influence was detected in homogenized raw milk. Further, the work of Davies (1), and Krukovsky and Sharp (8) indicates oxidative changes occur simultaneously with, or perhaps precede, normal lipase action, but in these studies on "homogenization" lipolysis, no oxidative changes could be detected by means of the peroxide determinations, even though large amounts of fat splitting had occurred.

The fact that formalin had no inhibiting effect on lipolysis in homogenized raw milk would indicate that the lipase action in this product is different from that of normal milk, since Herrington and Krukovsky (5) found that formalin markedly lowers the lipase action in normal milk. However, Tarassuk (12) reported a study of milk from one cow in which formalin did not influence the activity of the lipase. On the basis of their formalin studies, Herrington and Krukovsky (5) expressed the belief that there are two lipases in milk, one of which is not affected by formalin. Dorner and Widmer (2) had previously suggested the presence of two lipases, one of which is extremely heat labile and which produces a sharp, bitter taste and marked acidity changes. These findings may indicate that the lipase in homogenized milk is different from the one responsible for the major portion of lipolytic activity in normal milk. However, additional proof of this is needed before definite conclusions may be drawn.

Further results of this study show that increasing the NaCl content decreases the lipolysis, whereas the lipase activity is increased by increases in the storage temperatures. Heating of the cream and skim milk fractions indicates that the lipase agent follows the plasma phase. Further, the lipolysis in a prepared fat-skim milk product was greater than in a similarly prepared mixture of fat and whey, although the fat dispersed in the whey did undergo appreciable splitting.

CONCLUSIONS

Lipolysis in homogenized raw milk is not affected, in all cases, by the same factors which have been found to influence the rate of fat splitting in normal milk. Whether these variations are due to different lipases or whether merely due to physical or physico-chemical changes involving the fat globules has yet to be definitely determined.

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OXIDATION-REDUCTION POTENTIALS AND THE OXIDIZED FLAVOR IN HOMOGENIZED MILK*

P. B. LARSEN, I. A. GOULD AND G. M. TROUT

Department of Dairying, Michigan State College, East Lansing, Michigan

Various workers have observed that homogenization tends to stabilize milk against oxidative changes (2, 3, 7, 11, 12, 13), but the mechanism by which the stabilization is produced has not been definitely ascertained. Ross (7), Dahle (1), and Thurston (11) expressed the belief that the adsorbed layer around the fat globules is the protective agent involved in homogenization. Earlier, Tracy, Ramsey and Ruehe (12) indicated that certain physical changes in the milk were involved which might have made the oxidized flavor less detectable.

Efforts have been made to correlate oxidation-reduction potentials with oxidized-flavor development. However, the results secured by Thurston (9), Greenbank (6), Webb and Hileman (14), and Fox (4) indicate that the potential values were not a definite indication of the tendency of normal milk to become oxidized. More of a relationship between oxidation-reduction potentials and oxidized-flavor development would be expected when the oxidation is induced by copper since Tracy, Ramsey and Ruehe (12), Gebhardt and Sommer (5), Thurston (9), Webb and Hileman (14), and Swanson and Sommer (8), found copper to cause a rise in the potential.

Although the role of homogenization in the control of flavor has been previously studied, no consideration has been given to the oxidation-reduction changes which may occur in homogenized milk under different conditions. Consequently, this study was conducted with the view of ascertaining these changes.

EXPERIMENTAL PROCEDURE

Mixed-herd milk obtained from the College creamery was used in the major portion of these studies. Pasteurization was accomplished at 143–145° F. for 30 minutes in stainless steel equipment. The milk was homogenized at the pasteurization temperature and at 2500 pounds pressure with a new style commercial-size viscolizer. The milk was cooled at once and stored at 34–40° F.

When copper was used, it was added as a weak solution of copper sulfate following the pasteurization and homogenization of the milk.

Oxidation-reduction potentials were determined by means of a Beckman pH meter using a bright platinum wire electrode in circuit with a saturated calomel cell. Usually about fifteen to twenty minutes were required before constant results could be obtained.

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* Jour. Art. 516 (n. s.) from the Michigan Agricultural Experiment Station.

Organoleptic examinations were made by at least two experienced milk judges. The samples were numbered in such a manner that their identity remained unknown to the judges. The intensity of the oxidized flavor was indicated as follows: 0—no flavor; 1—questionable; 2—slight; 3—distinct; 4—strong.

EXPERIMENTAL RESULTS

Preliminary studies. A large number of preliminary trials were conducted in which milk was utilized which was normally susceptible to oxidized-flavor development. This milk was secured direct from the College farm. The results of these preliminary experiments showed the unhomogenized milk to become oxidized on storage, whereas the homogenized milk did not develop this defect. However, there was no significant difference in the oxidation-reduction (Eh) of the unhomogenized and homogenized milk, both of these milks tending to increase in potential during storage. These preliminary trials were the basis for additional studies.

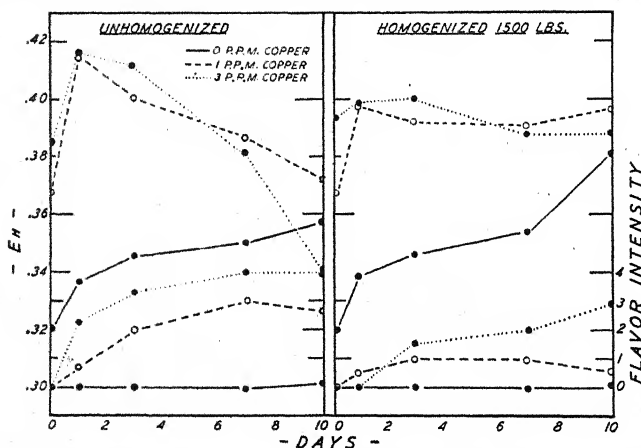


Fig. 1. Changes in oxidation-reduction potentials and flavor in unhomogenized and homogenized milk containing added copper. (Homogenization pressure 1500 pounds. Lower three curves represent flavor intensity.)

Oxidation-reduction potentials and oxidized flavor in copper-treated homogenized and unhomogenized milk. In these experiments trials were conducted in which the oxidized flavor was induced by the addition of copper to milk which was normally not susceptible to oxidation. Copper was added in concentrations of 0, 1, and 3 p.p.m. to homogenized and unhomogenized milk. Homogenization pressures used were 1500 and 2500 pounds.

The results of the trials in which the milk was homogenized at 1500 pounds pressure are shown in figure 1. Results are shown both for the unhomogenized and for the homogenized milk.

This figure shows the close similarity between the Eh values of the

unhomogenized and homogenized milk, irrespective of the differences in flavor changes. The lots which contained no added copper show a gradual change upward during the ten-day period. This occurred both in the homogenized and unhomogenized product. The addition of copper, either 1 p.p.m. or 3 p.p.m., markedly increased the potential of the milk. The most abrupt Eh rise in the copper-contaminated samples occurred during the first day; thereafter, the potential tended to decrease, with the decrease being especially noticeable in the unhomogenized milk.

From the standpoint of flavor, the unhomogenized milk to which copper was added developed distinct to strong oxidized flavors before the third day, with the 3 p.p.m. samples becoming oxidized within 24 hours. The untreated-unhomogenized milk remained practically free of oxidized flavor during this 10-day period. The untreated-homogenized milk, likewise, was free from oxidized flavor during the storage period, and the milk containing 1 p.p.m. of added copper showed only an extremely slight tendency towards oxidation. However, the 3 p.p.m. of copper were sufficient to overcome the stabilizing ability of 1500 pounds of homogenization as shown by the fact that the samples containing this amount of copper gradually developed a higher intensity of the oxidized flavor during storage. The oxidized flavor in the homogenized milk developed more slowly and to a lesser extent than in the unhomogenized milk similarly treated with copper.

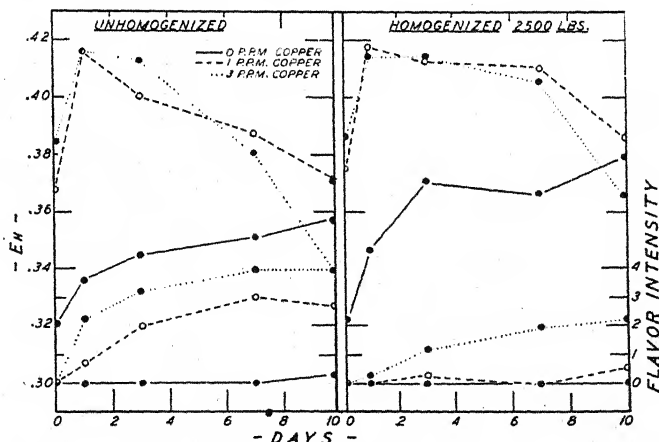


FIG. 2. Changes in oxidation-reduction potentials and flavor in unhomogenized and homogenized milk containing added copper. (Homogenization pressure 2500 pounds. Lower three curves represent flavor intensity.)

The results secured when 2500 pounds of pressure were used are illustrated by figure 2. These results are not greatly different from those in which 1500 pounds pressure was used. Again, the Eh values for the homogenized milk were similar to those for the unhomogenized, with the

copper causing marked increases in the potential in both cases. Flavor changes were also similar between these trials and those shown in figure 1. The 2500 pounds pressure was sufficient to prevent the development of the oxidized flavor in the presence of 1 p.p.m. of added copper but was unable to protect entirely the milk when 3 p.p.m. of copper were used. It should be emphasized in this connection that the copper was added following homogenization; thus the flavor stabilizing ability of the homogenization process is less than if the copper had been added prior to the pressure treatment (7, 13).

In general, the results illustrated in figures 1 and 2 show that oxidation-reduction potential changes are not definitely related to copper-induced oxidized flavor when homogenization is involved. The addition of copper does increase the potential in homogenized milk, whereas the oxidized flavor may or may not develop, depending on the protective power of the homogenization process.

SUMMARY AND CONCLUSIONS

Homogenization of milk tends to stabilize the milk against oxidation but has no influence on changes in the oxidation-reduction potentials. Trends in Eh were similar regardless of homogenization.

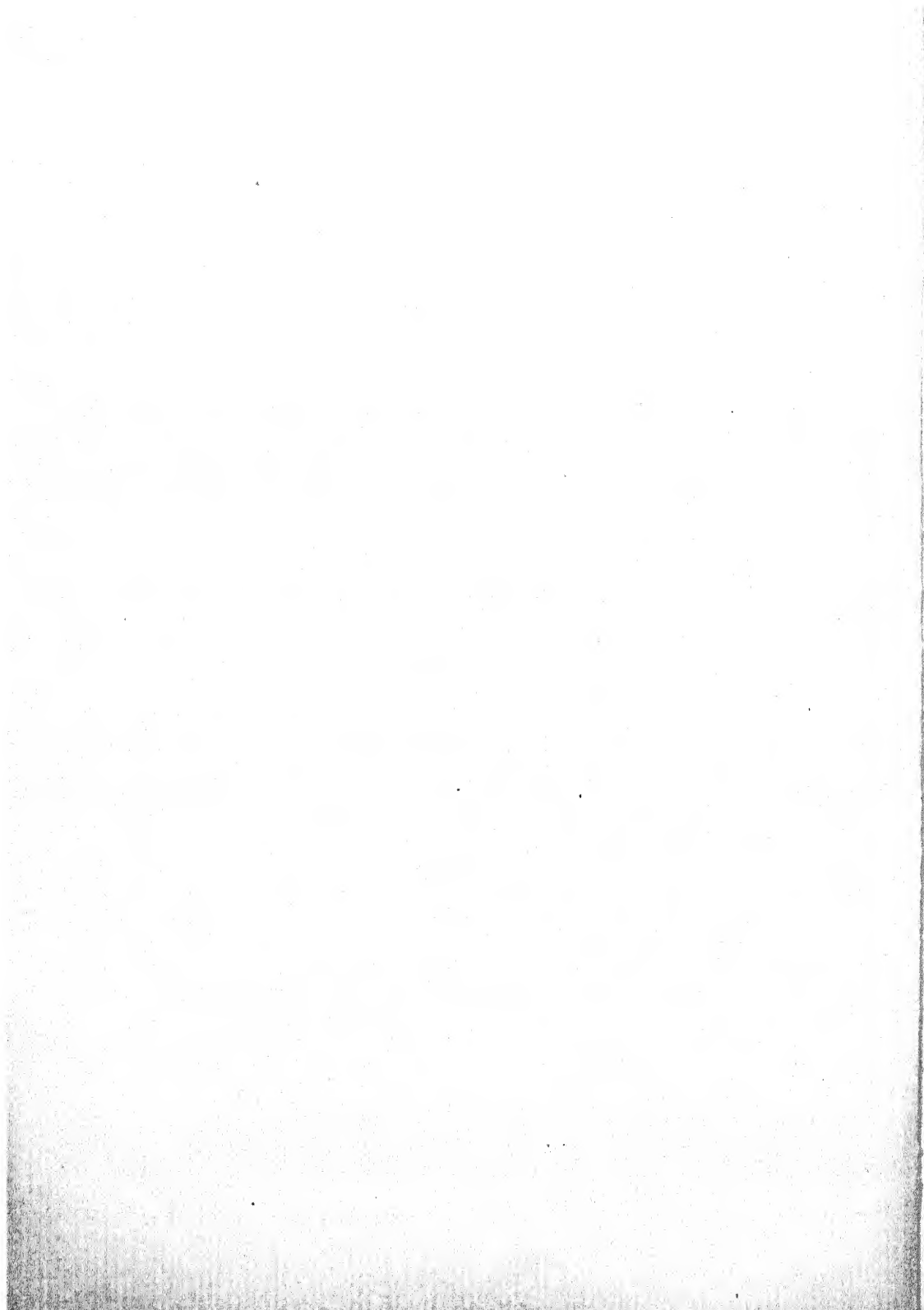
The mechanism by which homogenization prevents or retards oxidized-flavor development would appear not to be associated with oxidation-reduction potentials.

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LIVE WEIGHT OF COW AT VARIOUS STAGES OF LACTATION IN RELATION TO MILK-ENERGY YIELD

W. L. GAINES

Illinois Agricultural Experiment Station, Urbana, Illinois

In connection with the postulate previously advanced (1) that milk-energy yield per unit live weight is independent of live weight, it seems desirable to emphasize that the postulate is based on live weight at the start of the lactation and milk-energy yield for the first 8 months of the lactation. The 8-months feature avoids complications of advanced pregnancy, and probably affords as good a biological measure of dairy development as any longer period of the same lactation, even in farrow cows.

What is the relation of live weight to the 8-months yield, and how is it affected by the stage of lactation at which live weight is measured? In the present paper some published data by Dawson, Kopland and Graves (2)¹ are utilized in answer to the above question, particularly with reference to the effect of stage of lactation at which live weight is measured.

ANALYTICAL PROCEDURE AND RESULTS

Another way of expressing the above postulate is to say that yield is proportional to live weight, and the conformity of observed data to the postulate may be tested by fitting the equation, $FCM = aW^b$, (FCM = milk-energy yield in pounds of 4 per cent milk per day for the partial lactation; W = live weight of cow in pounds). If the exponent b , turns out to be 1, it indicates FCM is proportional to W ; if more than 1, large cows yield more per unit live weight than small ones; if less than 1, small cows yield more per unit live weight than large cows. Primary interest with the present data (2) however is to see how the exponent, b , is affected by stage of lactation at which live weight is measured. From the published data live weight is taken at the first month of lactation; the second month of lactation; and so on to and including the twelfth month of lactation, with finally live weight as an average of the 12 monthly weights.

The exponent, b , in the equation, $FCM = aW^b$, has been determined by fitting a straight line, $FCM = a' + b'W$ and approximating b as, $b = b'(\bar{W}/FCM)$, where the overscore indicates the mean.² The values of b thus derived are given in table 1.

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¹ The authors have generously supplied the monthly fat percentage data, supplementing their Table 11, which permit the computation of monthly milk-energy yields.

² On the theory that FCM/W is a fundamental criterion of dairy development, it is desirable to have FCM/W for individual cows and lactations. In case the individual FCM/W 's have been fitted with the equation, $FCM/W = a'' + b''W$, (as in 3, fig. 1), b in the power equation may be approximated as $b = 1 + b''(\bar{W}/FCM/W)$. The derivation of these approximations will be explained in a later paper.

TABLE 1

*Relation of live weight in pounds (W) at various stages of lactation to partial lactation milk-energy yield (FCM)**

Month of lactation	Live weight, W			Correlation, r_{WFCM}		Value of b in $FCM = aW^b$ †	
	Lowest	Highest	Average	10-mo.	8-mo.	10-mo.	8-mo.
1	1125	1399	1284	0.62	0.70	1.11	1.07
2	1140	1346	1236	0.64	0.67	1.47	1.32
3	1164	1374	1243	0.64	0.68	1.56	1.41
4	1172	1345	1250	0.66	0.67	1.70	1.49
5	1174	1334	1251	0.61	0.58	1.72	1.39
6	1133	1331	1250	0.52	0.54	1.21	1.07
7	1151	1342	1255	0.49	0.51	1.18	1.05
8	1156	1348	1261	0.25	0.25	0.71	0.60
9	1180	1369	1271	0.13	0.12	0.35	0.28
10	1207	1424	1290	0.32	0.34	0.69	0.63
11	1203	1469	1311	0.37	0.43	0.72	0.72
12	1261	1505	1343	0.30	0.38	0.60	0.64
Av.	1195	1342	1271	0.53	0.57	1.41	1.29

* This table is based on 11 records of 8 Holstein cows (2). The cows were fed exclusively on alfalfa hay throughout (consuming up to 48 pounds per cow per day). Because of deferred breeding the 10-months partial lactation FCM is included, in addition to the 8-months, since no cow carried a calf more than 169 days during the 10-months partial lactation. All cows were in the second or later lactations. The last line of the table deals with W as an average of the 12 monthly W's.

† The formula for deriving b, as given in the text, shows that in the power equation the exponent, b, is (approximately) the linear regression in terms of the means. The coefficient of correlation, r, is the linear regression in terms of the standard deviations. In the 8-months partial lactation, for example, the FCM series is identical for each of the 12 months, and any differences in the r/b ratios, as between months, must arise in differences in variability in live weight (σ_w/\bar{W}) as between months.

From the last column of table 1 it is observed that the exponent, b, in the power equation, is 1.07 where live weight is measured in the first month of lactation; reaches a high of 1.49 where live weight is measured in the fourth month of lactation; and reaches a low of .28 where live weight is measured in the ninth month of lactation. The relation of yield to live weight is greatly affected by the stage of lactation at which live weight is measured.

It will be noted that the coefficients of correlation between weight and yield follow a somewhat different cycle of changes than b. The highest correlation is found at the first month of lactation. On the whole it appears that if a single live weight determination is made at each lactation, the most desirable time is during the first month after calving. Furthermore, for practical use in the field (in distinction to experimental work), it seems that this one point of measurement in each lactation may be quite adequate.³

³ The postulate that FCM/W is independent of W (8-months FCM and first-month W) is set up as a philosophy of what may reasonably be expected as between dairy cows of varying sizes, rather than as a statement of what now prevails among dairy cows. While the present 11 records are too few in number to be at all conclusive, statistically, they do afford an instance of observation in which FCM/W , within herd and within breed,

SUMMARY AND CONCLUSIONS

Eleven records of Holstein cows are fitted with the equation, $FCM = aW^b$, in which W is live weight measured successively at monthly intervals during the lactation period, and FCM is milk-energy yield for the first 8-months of the lactation period. There is a vast difference in the resulting value of b (.28 to 1.49), according to the stage of lactation at which live weight is measured. FCM and W are most closely related where live weight is measured in the first month of lactation ($r = .70$).

The above records and other experimental evidence indicate that, within a dairy breed and within a herd (comparable environment) FCM is proportional to the 1.07 power of live weight, where live weight is measured in the first month of lactation. Both practical and biological considerations indicate the desirability of estimating live weight of the cow in the first month of each lactation in D.H.I.A. and similar milk-recording work. Measuring live weight in this way, it appears sound to measure lactation performance of the cow at each lactation in terms of FCM/W , that is, milk-energy yield for the 8-months partial lactation per unit live weight in the first month of the lactation.

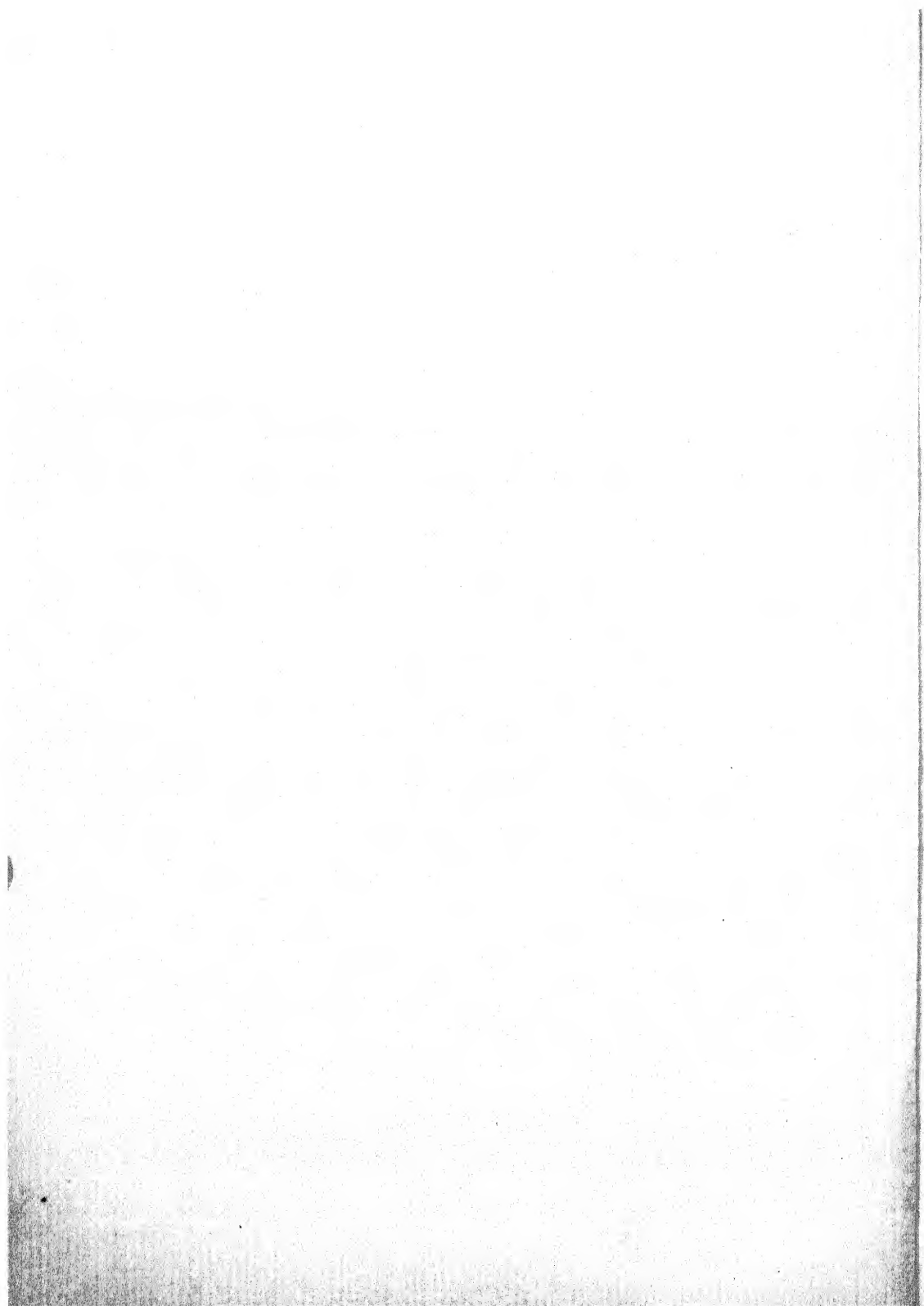
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is substantially independent of W , that is, referring to the observed fact that FCM is proportional to the 1.07 power of live weight.

Another similar instance is afforded by a previous analysis (3) of 66 records of Holstein cows (heavily grain fed) reported by the Cornell Station, including accurate initial live weight and partial lactation milk-energy yield for 280 or 259 days. From these records (3, fig. 1) applying the method of footnote 2, it is found that $b = 1.07$, as an average. That is, in these experimental records, also, FCM is proportional to the 1.07 power of live weight.

The only other records, based specifically on first-month weight and 8-months yield, known to the writer consist of a more numerous (1152) but less accurate body of D.H.I.A. records, in which it was found (4, footnote 3) that within herd and within breed (Holstein or Jersey) FCM was proportional to about the $\frac{2}{3}$ power of live weight. It now comes to light that the scale of the chest-girth live-weight tape used in estimating live weight was erroneous, grossly over-estimating the weight of large cows and grossly underestimating the weight of small cows. It appears likely that removal of this bias in the weight estimate would result in showing in these records also that FCM was proportional to the first-month live weight (rather than the $\frac{2}{3}$ power of weight).



THERMODURIC BACTERIA IN MILK. III. THE EFFECT OF CHANGING AGAR AND TEMPERATURE OF INCUBATION FOR PLATE COUNTS ON THE PROBLEM OF THERMODURIC BACTERIA IN MILK

J. L. HILEMAN, CLARENCE MOSS AND BETTY STEAD

Dairymen's League Co-Operative Association, Inc., Syracuse, N. Y.

In 1939 the American Public Health Association (1) adopted a much richer medium than had hitherto been used as the standard for plate counts of bacteria in milk. It has also been proposed that the temperature of incubation for the plates be reduced from 37° C. to 32° C. (2). Several investigations of the effect of the new medium on counts of various dairy products have been published (3, 4, 5, 6, 7, 8). These investigations do not clearly show whether the per cent increase in count is different in the case of raw milk than it is in the case of pasteurized milk, although several investigations on a medium similar to the new standard medium (tryptone glucose skim milk agar) indicate that pasteurized milk gives a much greater per cent increase in count than does raw (9, 10, 11). Similarly, the work of Kelly shows that lowering the temperature of incubation from 37° C. to 32° C. causes a greater per cent increase in count with pasteurized milk than with raw milk (12). These reports would seem to indicate that either enrichment of the agar or lowering of the temperature of incubation would tend to favor the growth of thermophilic bacteria. However, the comparisons available in the literature are not direct comparisons of counts on the same milk before and after pasteurization, and there is, in general, little known about the history of the samples. For that reason it seemed desirable to determine the effect of these variations in methods of enumerating bacteria on the counts on the same milk both before and after pasteurization, so that it would be possible to state clearly the effect of changing the method of counting on the number of thermophilic bacteria disclosed and on the per cent of the total organisms counted in the raw milk which are reported to be thermophilic.

EXPERIMENTAL

The work reported here was done in a bottling plant receiving approximately 70,000 pounds of milk daily from about 300 producers. One hundred lots of milk were examined. For each lot, about 300 gallons of raw milk were drawn into a glass-lined pasteurizer, and after agitation a sample was withdrawn in a sterile vial by dipping the vial (held in a clamp) into the milk. The milk in the pasteurizer was heated to 143°-144° F. (61.6°-62.2° C.), held for 30 minutes, and cooled in the pasteurizer to about 130°

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F. (54.4° C.). A sample of this pasteurized milk was withdrawn in a sterile vial, quickly cooled in ice water, and used for a bacteria count.

The raw milk samples were divided into three portions. One was used for a bacteria count while raw. A second was pasteurized in the laboratory at 143°–144° F. for 35 minutes. A third was pasteurized in the laboratory at 160.5°–161.5° F. (71.4°–71.9° C.) for 16 seconds. Heating to pasteurizing temperature required about 27 minutes in the plant pasteurization, 4 minutes in the laboratory low-temperature pasteurization, and 2.5 minutes in the laboratory high-temperature pasteurization. Cooling was very rapid in both laboratory pasteurizations, and cooling to 130° F. in the plant required about 5 minutes.

Four plates were made on each sample, or 1600 plates for the 100 lots of milk. Two of the four plates were poured with the old standard nutrient agar, and two with tryptone glucose extract milk agar, both media being made from Difco Dehydrated products. One plate with each agar was incubated at 37° C., the other at 32° C., the incubation period being very close to 48 hours. A Quebec Colony Counter was used in counting colonies. Adequate blanks were made to assure sterility of all materials.

The experiments covered a period from October, 1940, through January, 1941. Because of the large amount of space that would be required to show in detail each of the 1600 counts, averages were made of the counts on the

TABLE 1

Averages of bacteria counts per milliliter on 100 lots of milk when raw and after pasteurization by three methods, when determined on two agars and at two temperatures of incubation

Treatment of milk	Method of counting		Bacteria per milliliter	Per cent of count on old agar at 37° C.	Per cent of count on new agar at 32° C.	Per cent of raw milk count (or per cent of thermidurics)
	Designation	Description				
Raw	A	Old agar at 37° C.	72,830	100.0	57.4
	B	Old agar at 32° C.	101,230	138.9	79.8
	C	New agar at 37° C.	90,540	124.3	71.3
	D	New agar at 32° C.	126,820	174.1	100.0
Pasteurized in the plant at 61.6° C. for 30 minutes	A	Old agar at 37° C.	6,725	100.0	26.5	9.2
	B	Old agar at 32° C.	13,769	204.7	54.2	13.6
	C	New agar at 37° C.	15,045	223.7	59.2	16.6
	D	New agar at 32° C.	25,395	377.6	100.0	20.0
Pasteurized in the laboratory at 61.6° C. for 35 minutes	A	Old agar at 37° C.	4,806	100.0	21.9	6.5
	B	Old agar at 32° C.	11,577	240.8	52.7	11.4
	C	New agar at 37° C.	12,226	254.3	55.7	13.5
	D	New agar at 32° C.	21,932	456.3	100.0	17.2
Pasteurized in the laboratory at 71.6° C. for 16 seconds	A	Old agar at 37° C.	5,319	100.0	24.3	7.3
	B	Old agar at 32° C.	12,568	236.2	57.5	12.4
	C	New agar at 37° C.	12,512	235.2	57.2	13.8
	D	New agar at 32° C.	21,853	410.8	100.0	17.2

raw and the three kinds of pasteurized milk, as determined on the two agars and at the two temperatures of incubation. These average counts are shown in table 1 in the first or left-hand column.

The second column is designed to show the numerical relationship between the counts on the old agar at 37° C. and the counts by the other three methods of counting.

The third column is similar to the second, except that it shows the relationship between the count on the new agar at 32° C. and the counts by the other three methods of counting. If it is assumed that the count on the new agar at 32° C. gives the maximum possible count, or a count that approaches all of the organisms present (an assumption that is certainly not entirely justified), then the figures given in the third column represent the percentage of the total organisms present that will grow under the conditions specified.

The fourth or right-hand column shows the per cent of the organisms present in the raw milk, as determined by the four methods of counting, that will survive each of the three methods of pasteurization.

It is possible, on the basis of the counts made by the four methods, and designated in table 1 as A, B, C and D, to divide the organisms growing on the plates into four groups (shown in table 2 as classes 1, 2, 3 and 4), as follows:

1. Those that will grow on the old agar at 37° C. This is obviously the total count on the old agar at 37° C.
2. Those that require the temperature of incubation to be reduced from 37° C. to 32° C. but do not require enrichment of the agar. This is the difference between the count on the old agar at 32° C. and that on the old agar at 37° C. or (B-A).
3. Those that require enrichment of the agar but not reduction of the temperature of incubation. This is the difference between the count on the new agar at 37° C. and the count on the old agar at 37° C. or (C-A).
4. Those that require both enrichment of the agar and reduction of the temperature of incubation. This is a more complicated calculation, involving all four counts. It is done as follows:
 - a. If A, the count on the old agar at 37° C., is subtracted from C, the count on the new agar at 37° C., the result is the effect on the count of changing the agar at 37° C. This may be expressed at (C-A).
 - b. If B, the count on the old agar at 32° C., is subtracted from D, the count on the new agar at 32° C., the result is the effect on the count of changing the agar at 32° C. This may be expressed as (D-B).
 - c. If there are present any organisms requiring both that the

agar be enriched and the temperature of incubation be lowered before growth will occur, then $(D - B)$ will be greater than $(C - A)$, and the difference between these two values will give the number per milliliter of such organisms present.

- d. Then the number of organisms of class 4 is given by $(D - B) - (C - A)$, which is equivalent to $(D + A - B - C)$. Thus, the number of organisms of this class was determined by adding D and A , and from that sum subtracting both B and C .

Table 2 shows the result of making the calculations described in the preceding paragraph. The four columns of figures have the same significance as in table 1.

TABLE 2

Numbers per milliliter of four classes of organisms in 100 lots of milk when raw and after pasteurization by three methods, when determined on two agars and at two temperatures of incubation

Treatment of milk	Class number	Description of class of organism	Number per milliliter	Per cent of number growing on old agar at 37° C.	Per cent of number growing on new agar at 32° C.	Per cent of number in raw milk (or per cent of thermidurics)
Raw	1	Grow on old agar at 37° C.	72,830	100.0	57.4
	2	Require 32° C. but not new agar	28,400	38.9	22.4
	3	Require new agar but not 32° C.	17,710	24.3	14.0
	4	Require both new agar and 32° C.	7,880	10.8	6.2
Pasteurized in the plant at 61.6° C. for 30 minutes	1	Grow on old agar at 37° C.	6,725	100.0	26.5	9.2
	2	Require 32° C. but not new agar	7,044	104.7	27.7	24.8
	3	Require new agar but not 32° C.	8,320	123.1	32.8	46.9
	4	Require both new agar and 32° C.	3,306	49.1	13.0	41.9
Pasteurized in the laboratory at 61.6° C. for 35 minutes	1	Grow on old agar at 37° C.	4,806	100.0	21.9	6.5
	2	Require 32° C. but not new agar	6,771	140.8	30.9	23.8
	3	Require new agar but not 32° C.	7,420	154.4	33.8	41.8
	4	Require both new agar and 32° C.	2,935	61.0	13.4	37.2
Pasteurized in the laboratory at 71.6° C. for 16 seconds	1	Grow on old agar at 37° C.	5,319	100.0	24.3	7.3
	2	Require 32° C. but not new agar	7,249	136.2	33.2	25.5
	3	Require new agar but not 32° C.	7,193	135.2	32.9	40.6
	4	Require both new agar and 32° C.	2,092	39.2	9.6	26.5

DISCUSSION

Examination of the data in tables 1 and 2 would seem to justify the following conclusions:

1. Either lowering of the temperature of incubation from 37° C. to 32° C., or enriching the agar, or making both changes simultaneously, results in a higher bacteria count with either raw or pasteurized milk (table 1).
2. With either raw or pasteurized milk, changing both temperature of incubation and composition of the agar simultaneously results in a greater increase in count than making either change alone (table 1).
3. The per cent increase in count due to any of these changes in methods of enumeration is from two and one-half to five times as great with pasteurized milk as with raw milk (table 1).
4. The most logical explanation of this difference between raw and pasteurized milk would seem to be as follows:
 - a. Only a few of the raw-milk organisms that can grow on the old agar at 37° C. are capable of surviving pasteurization. The three methods of pasteurization used gave 6.5, 7.3 and 9.2 as the per cent of thermoduric bacteria among these raw-milk organisms of Class 1 (table 2).
 - b. Of the bacteria in raw milk which require that the temperature of incubation be reduced, or that the medium be enriched, or that both changes be made before growth is possible on the plates, relatively large percentages are thermoduric. The fourth or right-hand column of table 2 shows that, in the milk examined, there were from 24 to 46 per cent of thermoduric bacteria among these raw-milk organisms of Classes 2, 3 and 4.
5. The discussion immediately above means that changing from the old standard method of making plate counts in any one of the three ways studied results not only in higher total counts on both raw and pasteurized milk, but also results in a greater percentage of the organisms counted in the raw milk being classified as thermoduric. Thus, with all three methods of pasteurization used, the per cent of thermoduric bacteria (table 1) was more than twice as great when using the new agar at 32° C. as when using the old agar at 37° C.
6. The organisms requiring for their growth both that the old agar be enriched and that the temperature be reduced from 37° C. to 32° C. (Class 4 in table 2) are obviously capable of growing only under rather restricted environmental conditions. That being the case, it might be expected that they would not be as numerous as other organisms capable of growing under a wider range of conditions. It is

interesting to note (table 2) that organisms of Class 4 actually do form the smallest of the four classes in both the raw milk and the milk pasteurized by each of the three methods.

7. Laboratory high-temperature, short-hold pasteurization tends to give higher counts than does laboratory low-temperature, long-hold pasteurization (table 1).
8. As the method of enumeration is changed so as to give higher and higher counts, the difference in count between milk pasteurized by the two laboratory methods decreases.

There is in the literature evidence tending to support conclusions one to five above (9, 10, 11, 12), and also conclusion seven (13, 14, 15, 16, 17, 18, 19, 20, 21, 22). It seems probable, therefore, that these conclusions have a fairly broad applicability to many milk supplies, although an effective campaign to reduce to a very low point the number of thermoduric bacteria in a given milk supply might alter the picture somewhat. Any idea as to how broadly conclusions six and eight could be applied to other milk supplies must await further investigation by other workers and with other milk supplies.

It should be pointed out that the calculations on which table 2 is based imply the assumption that there were in the samples no organisms growing at 37° C. but not at 32° C., or growing on the old but not on the new agar. That assumption, of course, is not entirely justified, but how many such organisms occurred in the samples examined could not be determined by the methods used.

The control and elimination from milk supplies of thermoduric bacteria is costing the dairy industry and the milk producer large sums of money. The problem assumes even greater magnitude when Departments of Health lower the number of bacteria they will allow in pasteurized products, as has been done recently in several localities. To illustrate this, a reduction during 1940 in allowable bacteria count in pasteurized milk from 50,000 to 30,000, or a decrease of 20,000, appears to be a reduction of 40.0 per cent. However, if this is compared with June, 1939, before the new agar became official, the picture is considerably changed. Table 1 shows, for the average of 100 comparisons on commercially pasteurized milk, an increase of from 6,725 to 15,045 bacteria per milliliter, or 123.7 per cent in changing from the old to the new agar at 37° C. At this rate, a sample that would have shown a count of 50,000 bacteria per milliliter in June of 1939 would have a count of 111,850 at present. Therefore, enforcement of a standard calling for a maximum of 30,000 per milliliter at the present time actually means a reduction of 73.1 per cent as compared with two years ago. If the temperature of incubation is also changed, another increase in count, of even greater magnitude, will occur, which will increase still more the difficulty and expense of keeping the bacteria count of pasteurized milk at very low levels.

SUMMARY

Data on 100 lots of milk examined both before and after pasteurization shows that lowering the temperature of incubation for plate counts from 37° C. to 32° C. or changing from the old standard agar to tryptone glucose extract milk agar, or making both changes simultaneously, not only results in higher counts on both raw and pasteurized milk, but also results in a higher percentage of the organisms counted in the raw milk being classified as thermoduric. Three different methods of pasteurization all gave this same result.

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THE DETERMINATION OF FAT IN THE PRESENCE OF FREE FATTY ACIDS. II. DIFFERENCES IN THE BEHAVIOR OF INDIVIDUAL ACIDS IN THE MOJONNIER TEST

MORTIMER P. STARR

Brooklyn College, Brooklyn, New York*

AND

B. L. HERRINGTON

Cornell University, Ithaca, New York

In a previous report (8) we demonstrated that approximately 24 per cent of a mixture of fatty acids which resembled completely hydrolyzed butterfat was extracted in the Mojonnier test and determined as butterfat. This substantiated earlier reports (1, 4) of low Mojonnier fat tests in rancid dairy products, and indicated, also, that not all of the free fatty acid was retained in the ammoniacal layer. In those experiments, we used a mixture of free fatty acids which resembled that which would have resulted from the hydrolysis of butterfat by a *non-specific* lipase. Davies (2) states that "... the lipases are ... specific in their action ... thus lipases from various sources will show different rates of liberation of free fatty acids and a different distribution of such acids, although it is recognized that it is the unsaturated acids, *e.g.*, oleic acid, which are liberated in greatest amount. ...". For this reason, data on the recovery of individual fatty acids would be of some value in interpreting the decrease in fat test when examining rancid samples by the Mojonnier method.

EXPERIMENTAL

Quantities of a butter oil, which had been prepared from a fresh, high quality, unsalted, sweet-cream butter, and which contained 99.1 per cent of Mojonnier-extractable fat, were weighed into Mojonnier flasks. To each flask was added a weighed amount of *one* of the following fatty acids: butyric, lauric, myristic, palmitic, stearic, oleic. The stoppered flasks were then warmed in a water bath to melt the contents, shaken to mix the fat and acid, and then examined for "fat content" by means of the conventional Mojonnier test for butter (5). The data obtained in this manner are shown in table 1.

These data confirm our previous report that a certain fraction of free fatty acid is extracted and determined as butterfat when rancid butterfat is examined by the Mojonnier method for fat.

The quantity of free fatty acid which is extracted depends upon the nature of the fatty acid. Undoubtedly a number of more-or-less obvious

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TABLE 1

Mojonnier analyses of samples consisting of butter oil plus a free fatty acid

No.	A	B	C	D	Fatty acid not recovered		G
	Fat. Corrected weight of Mojonnier extractable fat in but- ter-oil	Fatty acid	Total sample (A + B)	Recov- ered by extrac- tion			Average
					(C - D)	$\frac{E \times 100}{B}$	
	mg.	mg.	mg.	mg.	mg.	%	%
Oleic							
1	472.9	0.0	472.9	472.9			
2	379.0	107.6	486.6	408.8	77.8	72.3	
3	339.9	160.0	499.9	384.0	115.9	72.4	
4	195.5	271.6	467.1	269.3	197.8	72.8	
5	0.0	909.0	909.0	128.0	781.0	(86.0)*	72.5
Stearic							
6	349.4	0.0	349.4	349.4			
7	279.5	29.7	309.2	285.7	23.5	79.1	
8	387.4	60.8	448.2	400.9	47.3	77.8	
9	190.6	220.5	411.1	239.7	171.4	77.7	
10	0.0	177.1	177.1	38.6	138.5	78.2	78.2
Palmitic							
11	195.6	0.0	195.6	197.5	
12	469.4	0.0	469.4	470.0	
13	375.8	67.1	442.9	390.4	52.5	78.2	
14	200.0	66.5	266.5	215.1	51.4	77.3	
15	275.0	114.3	389.3	302.4	86.9	76.0	
16	363.3	157.5	520.8	403.2	117.6	75.3	
17	256.2	177.3	433.5	297.8	135.7	76.5	
18	0.0	121.9	121.9	28.2	93.7	76.9	76.7
Myristic							
19	352.0	0.0	352.0	352.0	
20	286.8	57.7	344.5	291.4	53.1	92.0	
21	182.8	311.7	494.5	208.0	286.5	91.9	
22	0.0	223.4	223.4	1.0	222.4	(99.6)*	92.0
Lauric							
23	278.2	0.0	278.2	278.2			
24	345.1	25.5	370.6	346.7	23.9	93.7	
25	316.7	119.6	436.3	325.3	111.0	92.8	
26	358.9	152.8	511.7	368.6	143.1	93.7	
27	0.0	169.6	169.6	1.2	168.4	(99.3)*	93.4
Butyric							
28	369.5	67.6	437.1	369.3	67.8	100.3	
29	0.0	182.3	182.3	0.6	181.7	99.7	100.0

* Values in parentheses are excluded from the averages for reasons given in text.

factors are instrumental in causing these differences. Certain of these factors have been studied and will be discussed in turn; *viz.*, the hydrolysis of easily-dissociated ammonium soaps, the extraction of fatty acids thus liberated, and the loss of these extracted acids by volatilization.

The presence of ammonia in the ether extracts *before* evaporation may be demonstrated by means of Nessler's reagent. However, since ammonia is found, also, in the ether extract of a lipid-free control, the ammonia is not necessarily extracted in the form of an ammonium soap, and it is probable that the positive Nessler test may be traced to the extraction of some ammonia by the ether. Since no ammonia can be found in the lipid residue *after* evaporation of the ether, the volatile ammonia is probably driven off when the ether is evaporated. In this connection, one should not ignore the possibility that the ammonium soap was extracted but was decomposed by the heating afterward. Even $(\text{NH}_4)_2\text{SO}_4$ is reported to lose ammonia at 120°C . and, in some cases, even as low as 80°C . (7).

Since the residues in the fat dishes do not contain ammonia, it is evident that the fatty acid increment is free fatty acid and not the ammonium soap. This would be expected since these soaps are salts of the weak base, ammonium hydroxide, and the weak fatty acids. Extensive hydrolysis probably occurs in aqueous solution with the liberation of free fatty acids.

Indirect evidence for such an hydrolysis might be obtained by comparing the behavior of ammonium hydroxide with the behavior of a much stronger base. Lithium hydroxide seemed suitable for this comparison. It is a much stronger base than ammonia (the normal solutions are dissociated to the extent of 63 per cent and 0.4 per cent respectively (3)); and it yields soaps which are insoluble in ether but quite soluble in methyl alcohol (6).

These two bases were compared by running a series of analyses of cottonseed oil-oleic acid mixtures. The ammonia method yielded high results (approximately 27 per cent of the oleic acid was recovered), the lithium method gave the theoretical value for neutral fat alone. This difference is attributed to differences in the degree of hydrolysis of the respective soap solutions rather than to differences in the solubility of the soaps because we were unable to detect the presence of ammonia in the extracted lipid residues by means of Nessler's reagent.

We do not, however, advocate the substitution of lithium hydroxide for ammonium hydroxide in the Mojonnier test because troublesome emulsions may be formed and because the strongly alkaline lithium hydroxide may cause the saponification of some kinds of fat.

A further study of the data of table 1 shows that the recovery of fatty acid in the Mojonnier test varies with the molecular weight of the acid. This may be explained, in part, on the basis of differing distributions between the two solvents, and to differing volatilities of the extracted fatty acids. The solubility of fatty acids in water decreases with increasing

molecular weight. It should be expected, then, as is shown in table 1, that in the distribution of these acids between water and ether, a greater percentage of the acids of high molecular weight will be extracted by the ether than of acids of low molecular weight.

The acids of low molecular weight are more volatile than those of high molecular weight. The influence of volatility in reducing the recovery of certain free acids was shown by the following experiments:

Quantities of fatty acids and of butter oil were weighed directly into tared fat dishes; 50 ml. of ethyl ether and 50 ml. of petroleum ether were added and mixed with the lipids. The mixture was then heated on the Mojonnier fat plate to drive off the ethers, dried in the fat oven, cooled and weighed as in the conventional Mojonnier procedure. Some data obtained in this manner are recorded in table 2.

TABLE 2

The loss of fatty acid by volatilization during evaporation of the ethers in the Mojonnier fat test

No.	A	B		C	D	E	F
	Fat. Dry weight of butter oil	Fatty acid		Total sample (A + B)	Recovered after evaporation of ether	Fatty acid not recovered	
		Name	Weight			(C - D)	$\frac{E \times 100}{B}$
	mg.		mg.	mg.	mg.	mg.	%
101	555.0	0.0	555.0	555.1
102	419.7	Oleic	43.8	463.5	458.4	5.1	12.0
103	419.7	Oleic	143.0	562.7	560.1	2.6	2.0
104	0.0	Oleic	194.1	194.1	168.2	25.9	13.0
105	443.3	Stearic	150.1	593.4	593.1	0.3	0.2
106	0.0	Stearic	145.1	145.1	143.8	1.3	1.0
107	421.0	Palmitic	41.1	462.1	459.0	3.1	8.0
108	0.0	Palmitic	52.3	52.3	30.7	21.6	42.0
109	494.7	Myristic	225.8	720.5	716.1	4.4	2.0
110	0.0	Myristic	217.8	217.8	193.3	24.5	11.0
111	486.5	Lauric	76.7	563.2	542.7	21.5	28.0
112	0.0	Lauric	51.1	51.1	0.0	51.1	100.0
113	0.0	Butyric	182.3	182.3	0.6	181.7	100.0
114	0.0	0.0	0.0	0.0

These data show that the recovery of fatty acids in the Mojonnier test is influenced by the volatility of the acid. They also show that the volatility of the acids is reduced considerably by the presence of neutral fat. This probably accounts for the apparent discrepancies recorded in table 1; i.e., the high results in "amount of fatty acid not recovered" from samples 5, 22 and 27. In each of these cases, no neutral butterfat was present in the original sample. In those samples, a proportionately greater quantity of the extracted free acid was volatilized during the ether evaporation and, in this manner, a greater quantity of the fatty acid was "not recovered."

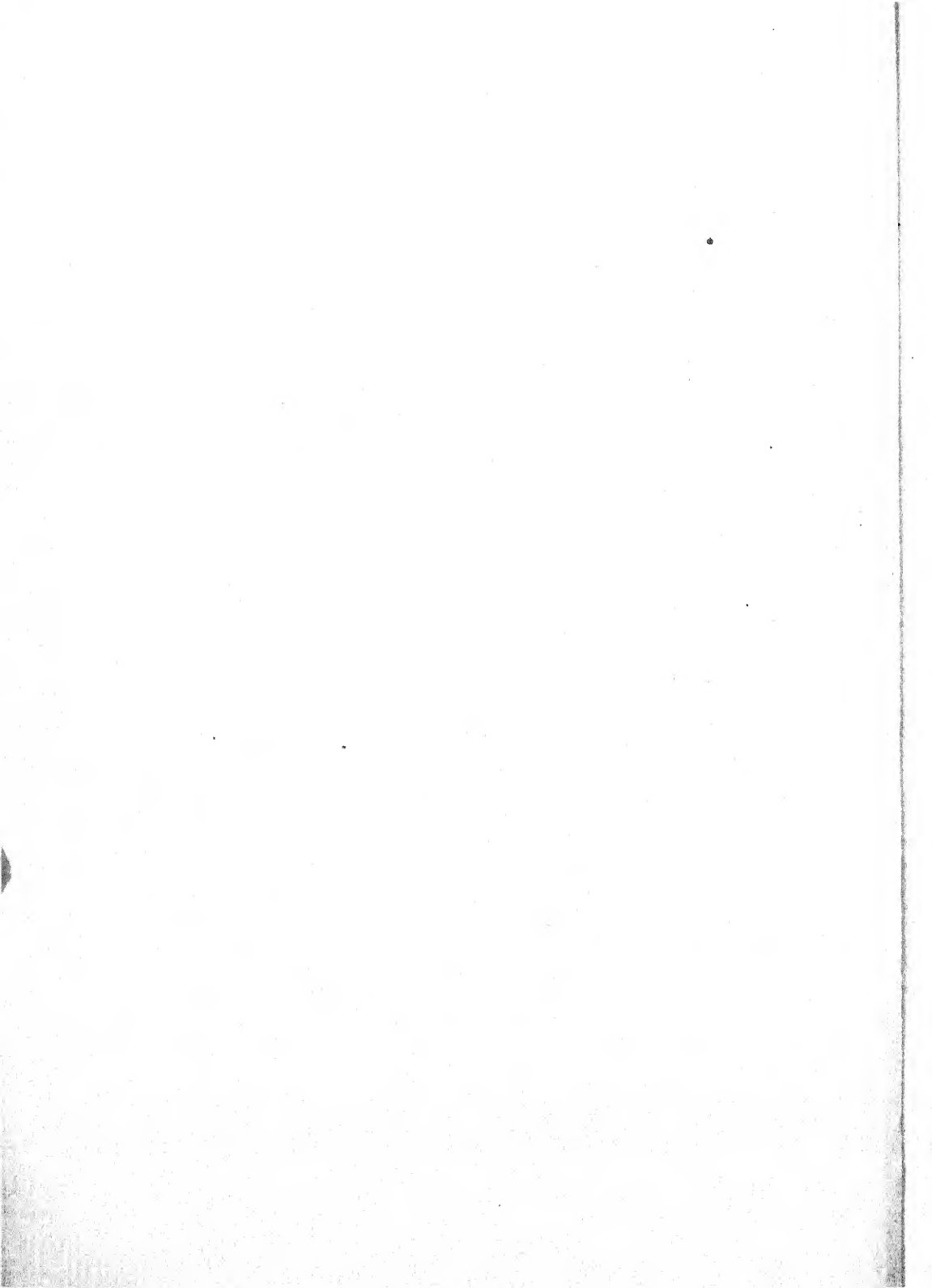
SUMMARY

The Mojonnier test does not recover equal percentages of the various free acids which may be present in the butterfat. This is due, in part at least, to variations in the amount of different acids volatilized when the sample is heated to remove the ether.

It seems probable that the recovery of free acids by the Mojonnier method can be traced to hydrolysis of the ammonium soaps with the subsequent extraction of the liberated acid. The degree of hydrolysis would depend upon the nature of the acid, but it might be reduced in all cases by the substitution of a stronger base for the ammonia. Lithium hydroxide offers some promise, but its saponifying action must be studied more carefully before it can be recommended.

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BUTTERFAT AND SILAGE CAROTENOIDS*

B. CONNOR JOHNSON, W. H. PETERSON AND H. STEENBOCK

Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison

Virtanen (12), Uuranen (11) and Peterson *et al.* (7, 8) observed that the apparent carotene content of silage prepared with acids was in many cases greater than that of the fresh material from which it had been made. The reason for these high values was found by Quackenbush, Steenbock and Peterson (9) to be the presence of pigments produced from xanthophyll by the action of silage acids. These pigments were carried along with carotene in the petroleum ether-ethanol procedure but could be separated from the carotene and from each other by chromatographic means. In some silages they made up 40 per cent of the so-called carotene, but unlike carotene they had no vitamin A potency.

It was the object of the present experiments to determine the extent to which the non-carotene pigments in "acid silages" would appear in butter produced from the milk of cows fed these silages. If these pigments were secreted into milk, they would be calculated as carotene and would give a false value to the vitamin A potency of the milk.

It was observed by Palmer and Eckles (6) that both carotene and xanthophyll occurred among the pigments of butter, but by far the greatest portion of the total pigment was carotene. Karrer and Schöpf (5) identified chromatographically lutein and zeaxanthin as well as carotene in butter. Gillam and Heilbron (3) found β -carotene and small amounts of α -carotene, kryptoxanthin and lycopene in the butter fat from cows which had received rations containing these pigments.

It is evident as stated by Strain (10) that the carotenoids of butter are dependent upon those in the ration of the cow, but they are present in different proportions in butter probably because carotene is much more readily absorbed than the other pigments.

EXPERIMENTAL

Butter was made in the winter of 1939 and again in 1940 from the milk of two groups of cows which had received respectively phosphoric acid alfalfa silage and molasses alfalfa silage for four months. Butter produced on summer pasture was used each year as a control. The butter samples were stored at a temperature below 0° C. and when required for analysis were melted and freed from water and solid matter by filtering through absorbent cotton. They were saponified under nitrogen, and the non-

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saponifiable matter was extracted with peroxide-free ether. The ether solution was washed several times with ice water, dried by freezing out the water with dry ice, evaporated to dryness in vacuum, and the residue taken up in 2 to 3 cc. of purified petroleum ether (Skelly-solve B). The solution was kept cool at all times and evaporated under vacuum in order to minimize isomerization as reported by Carter and Gillam (1) and by Zechmeister and Tuzson (13). The resultant solution was chromatographed through CaCO_3 or MgO columns. In most of the work CaCO_3 proved to be superior.

The pigments were fractionated as follows: The petroleum ether solution was forced into a uniformly packed column of adsorbent by means of air under a pressure of 15–25 pounds. The column was then washed with petroleum ether under continued pressure until the bands of carotene, acid-formed pigments and xanthophylls had separated. The bands of carotene and acid-formed pigments were washed through and collected separately. Then the xanthophyll bands were washed through with a 10 per cent solution of absolute alcohol in petroleum ether and collected together. The pigments were determined quantitatively in three groups: 1) carotene, 2)

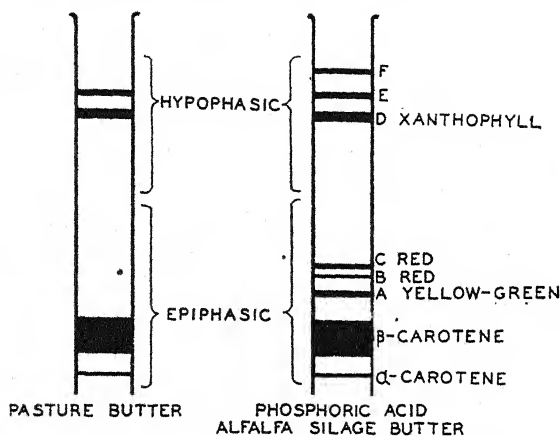


Fig. 1. Chromatograms of carotenoid pigments in butter fat.

pigments A, B, C (the so-called acid-formed pigments, Fig. 1), and 3) pigments D, E, F (the hypophasic xanthophyll pigments). The total pigment was first calculated as carotene by determining the intensity of spectral absorption in petroleum ether solution with a 440 m μ filter in the Evelyn photometer. After chromatographing, each fraction was read at 440 m μ in petroleum ether and expressed as carotene. The sum of these values was found to account for 95 to 97 per cent of the original values. Only the pigments which it seemed most important to identify were separated from the mixture.

To reveal the phasic distribution of the pigments a petroleum ether solu-

tion of the non-saponifiable fraction was extracted with 90 per cent methanol and the resulting two fractions were chromatographed from petroleum ether solution as before. In the case of the silage butters the carotene and the acid-formed pigments were found to be epiphasic while the xanthophylls were hypophasic.

RESULTS

The pigments which had been isolated chromatographically were identified so far as possible by determining their absorption maxima. The spectra were photographed in either petroleum ether or carbon bisulfide, with the use of a Hilger spectrograph. The wave lengths of the band maxima are given in table 1. The red acid-formed pigments (pigments B and C, Fig. 1) did not give distinct bands even in concentrated solution. In both petroleum ether and in carbon bisulfide, absorption was general over a wide range and the values given are, therefore, only approximate. It is possible that these pigments are still mixtures of degradation products.

TABLE 1
Absorption maxima of pigments isolated from butter

Pigment	In petroleum ether (m μ)	In carbon bisulfide (m μ)
α -Carotene { isolated	477, 444, 416	
{ accepted value	478, 447.5	
β -Carotene { isolated	483, 452, 425	519, 482, 450
{ accepted value	483.5, 452, 426	521, 485.5, 451
Yellow-green, acid-formed, pigment A	454, 428, 403	489, 454, 428
Red, acid-formed, pigment B	(495, 459, 425) ?	
Red, acid-formed, pigment C	(453, 433) ?	(480) ?
Xanthophyll { isolated		508, 473, 445
{ accepted value		508, 475, 445

Traces of α -carotene were found in the butters only when relatively large amounts of butter (100 gms.) were used in one analysis. This finding agrees with that of Gillam and El Ridi (2) who reported the α -carotene content to be less than 0.3 per cent of the β -carotene content in the butters which they examined.

The amounts of the pigment in each of the three groups for the phosphoric acid and the molasses alfalfa silage butters and for pasture butter are given in table 2. These figures are averages of the values obtained in 1939 and in 1940. From this table it appears that: 1) There is more carotene in pasture butter than in silage butter because of the higher carotene content of the pasture. However the ratio of carotene in butterfat to that in forage shows that carotene is absorbed just as well from silage as from pasture. 2) There are no acid-formed pigments in pasture and thus none

in pasture-butter. There are, as shown by Quackenbush *et al.* (9), more acid-formed pigments in acid silage than in molasses silage and, as would be expected, there are more acid-formed pigments in butter produced on phosphoric acid silage than on molasses silage. 3) While the acid-formed pigments seem to be rather readily absorbed and transferred into the butter-fat, the xanthophyll pigments appear to be so poorly absorbed that there is about the same amount of xanthophyll pigments in butters irrespective of the amount in the ration.

TABLE 2
Amounts of pigments found in butter (2 year averages)

Type of butter	Pigments in butter fat				(b) Carotene in forage	Ratio a/b
	Total	Xantho- phylls	Acid- formed	(a) Carotene		
	$\gamma/gm.$	$\gamma/gm.$	$\gamma/gm.$	$\gamma/gm.$	$\gamma/gm.$	
Pasture	11.0	2.2	0.0	8.8	250	0.035
Molasses						
alfalfa silage	9.0	2.2	0.7	6.1	140	0.043
Phosphoric acid						
alfalfa silage	8.5	2.0	1.0	5.5	120	0.046

Table 3 gives the distribution of the three groups of pigments expressed as per cent of the total pigments. In an analysis of the phosphoric acid alfalfa silage butter the 12 per cent of the acid-formed group was found to be made up of 3.4 per cent of the greenish-yellow pigment A and 8.6 per cent of the red pigments B and C.

TABLE 3
Distribution of pigments in butter (2 year averages)

Type of butter	Percentage of total pigments		
	Carotene	Acid-formed pigments	Xanthophylls
	%	%	%
Pasture	80	0	20
Molasses alfalfa			
silage	68	8	24
Phosphoric acid			
alfalfa silage	65	12	23

In order to obtain true values for the carotene content of milk the non-carotene pigments should be removed before the reading is taken. It would appear that the diacetone alcohol method of Hegsted *et al.* (4) might be applicable to milk and butter as well as to silage for the separation of the carotene and acid-formed pigments.

The above data should not be interpreted as denying the value of these forages for increasing the carotene content of winter milk, since only a part of the increased color is due to non-carotene pigments. Although no data

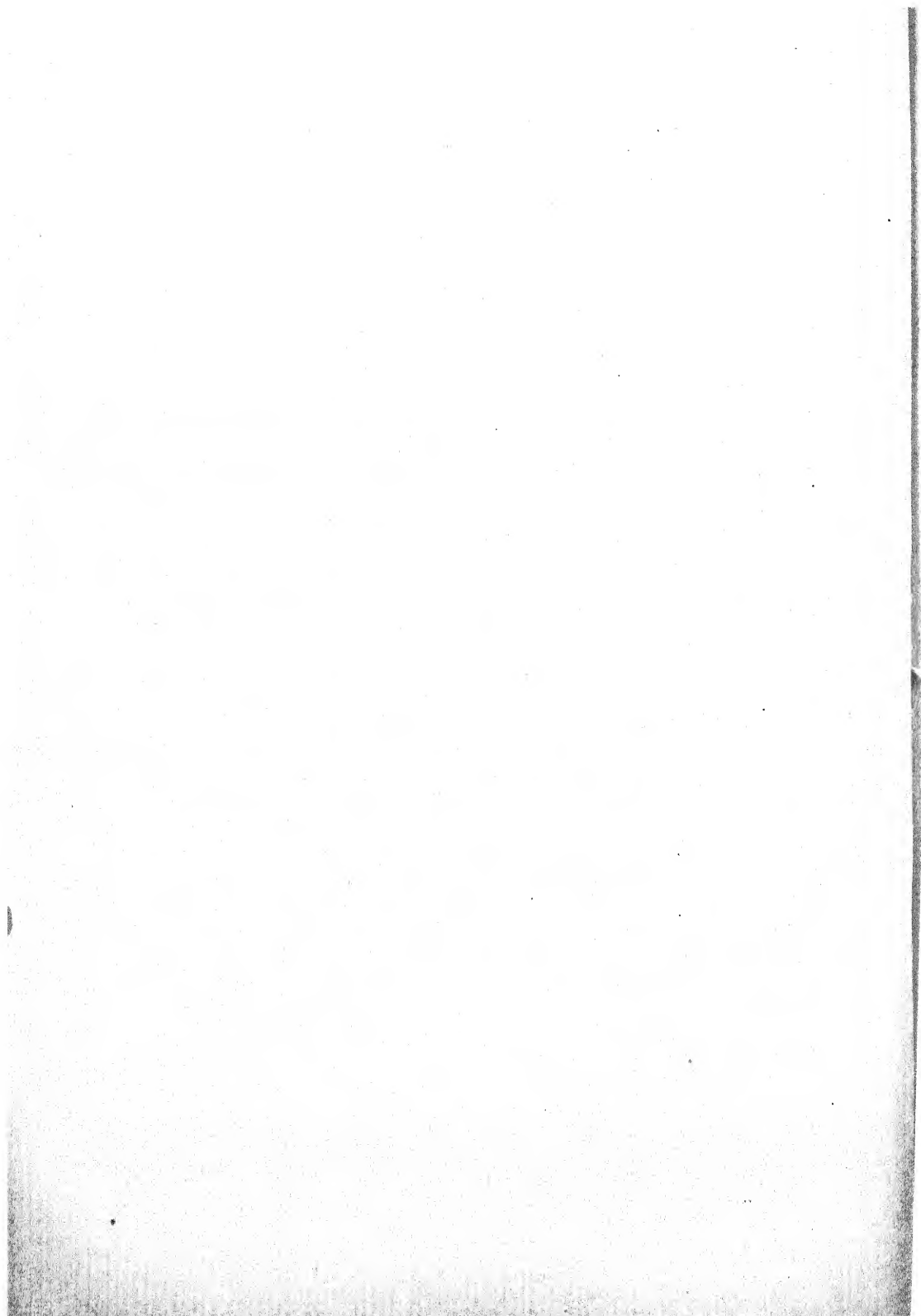
were obtained for butters produced by cows on hay-corn silage rations, it is probable that the percentage of non-carotene pigments in such butters would be as high as when the cows are fed molasses silage. Corn silage usually has a lower pH than molasses silage and hence a larger proportion of acid formed pigments would probably be present and presumably transmitted to the milk.

SUMMARY

Besides carotene and xanthophyll, pigments formed by the action of acids in silage are carried over into the butter fat of the milk. The usual methods of carotene analysis on butter fat do not distinguish between carotene and non-carotene pigments. By chromatographic methods the distribution of pigments in butters from cows on different forages was found to be as follows: pasture, 80 per cent carotene, 20 per cent non-carotene; molasses alfalfa silage, 68 per cent carotene, 32 per cent non-carotene; phosphoric acid alfalfa silage, 65 per cent carotene, 35 per cent non-carotene.

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MENSTRUATION FREQUENCY AND ITS RELATION TO CONCEPTION IN DAIRY CATTLE¹

GEORGE W. TRIMBERGER

Dairy Husbandry Department, University of Nebraska, Lincoln

Among mammals, there is a marked variation in regard to menstruation and its relationship to conception. Examination of the literature reveals that the time in the sexual cycle at which menstruation takes place is well established in the different species but there is a marked individuality that is observed for each one and sometimes even within the species.

The human being (1, 7) is reported to have an exceptionally long menstruation period which lasts from two to eight days with an average of five days. If the occurrence is regular, the beginning of the menstrual period is half way between successive ovulations and is closely associated with estrus. Marshall (5), Howell (4), Schmaltz (7), and Reynolds (6), all agree that in the human, the mucous membrane thickens to several times normal as pre-menstrual congestion takes place. Although menstruation in the human being is a phenomenon of the uterus, and blood will escape only from the surface of that organ, the other reproductive organs share to some extent the vascular congestion exhibited by the uterus during this period. The congested capillaries of the uterus break down or rupture in the superficial regions of that organ. The blood discharged at menstruation may be due to these small capillary extravasations and also to a process of diapedesis or seepage made possible by the congestion. Sometimes, when menstrual flow is very profuse in the human, there may be a considerable loss of surface epithelium. The vessels in the deeper tissue remain intact and none of the fluid is found free in the deeper tissue of the stroma. Howell (4) states that menstruation is a sign that fertilization has not taken place from the previous ovulation but Schmaltz (7) gives evidence that it is possible to have a certain amount of menstrual flow following fertilization.

Usually the experienced dog breeder knows that a bitch will, in most cases, have a pronounced flow of blood during the period of proestrus which usually lasts about ten days. As a rule, the bitch will not take the dog until bleeding has ceased and the best time for successful coition is soon after the cessation of this flow. Marshall (5) and Schmaltz (7) agree with this common view.

According to Marshall (5) occasionally blood has been observed in the mare's proestrus discharge but is not generally present. He also states that the ewe menstruates very little and usually shows no external signs. Very

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rarely, a small amount of bloody mucous is observed during proestrus. The sow likewise usually does not show any external signs but occasionally a bloody mucous flow has been observed during proestrus.

The cow (2, 3, 9) is reported to menstruate about two days after heat. Hammond (3) presents evidence to show that in the cow the menstrual fluid comes from both the vagina and uterus. He expresses the opinion that the blood flow in the vagina comes from the region situated just above the urethra and that in the uterus there is special engorgement of the placental areas; but bleeding can come from the surface of any part of these two organs.

A few authors express the opinion that if a heifer or cow menstruates a few days after service it is an indication that conception did not take place, but in most cases they present no definite data to support their belief. W. W. Williams (9) states "The non-pregnant cow ordinarily menstruates about the 48th hour after heat subsides. A little blood-tinted mucus stringing from the vulva at this time, or upon the cow's tail, indicates that the service has been ineffective. The complete absence of any bloody discoloration of the vaginal mucus suggests that the cow has conceived especially if it has previously been noted that the cow menstruated normally at other periods."

Another author, W. L. Williams (8), writes, "Within 24 to 48 hours after a cow has been bred she may menstruate. The sanious discharge emanating from the vulva may adhere to that organ, the tail and adjacent parts. If the cow has been bred and conceived, it is doubtful if there will be menstruation following. If she fails to conceive, menstruation is quite certain to occur. In many cases of serious sterility, the volume of menstrual blood is very great. Fertilization appears to inhibit menstruation, but menstruation may occur in spite of conception. The absence or presence of menstruation must not be accepted as final proof of conception or non-conception. It is, however, a valuable sign, and should always place the breeder and veterinarian on guard, with a rather definite expectation that the animal which has menstruated after breeding will again be in estrus in due course of time."

Hammond (3) carefully observed four heifers after service to a fertile bull and states that all four menstruated at the normal time a few days later. Three of these had become pregnant and subsequent bleeding did not occur in these three. He refers to the fact that it is a common belief among herdsmen that bleeding does not occur two days after heat if fertilization takes place.

EXPERIMENTAL PROCEDURE

Because of the widespread belief among livestock men that menstruation in cattle occurring after service is an indication that conception did not take place, and because of the conflicting opinions expressed in the litera-

ture, it seemed that the problem was worthy of consideration. As a result, data have been collected in the University of Nebraska dairy herd as to the occurrence of menstruation following estrus in heifers and cows. The study covered the years 1937 to 1940 and included representatives of the Jersey, Guernsey, Holstein, and Ayrshire breeds. As used in this study, menstruation refers to the external discharge of a bloody fluid, usually mucus mixed with blood, from the genital tract following estrus.

A group of 100 heifers and a group of 100 cows were studied to determine the frequency of the occurrence of menstruation following estrus when they were not served. For comparison, two similar groups consisting of 100 cows and 100 heifers were inseminated during estrus and the frequency of subsequent menstruation and conception recorded. The amount of menstrual fluid discharged was also noted and given a rating of slight, moderate, and pronounced. Observations were made at six regular intervals each day for five days following estrus and any external signs of a blood-tinted discharge were noted.

PRESENTATION OF RESULTS

Table 1 presents data on the occurrence of menstruation following estrus in open and bred heifers and cows and its relation to conception.

TABLE 1

Occurrence of menstruation following estrus in open and bred heifers and cows and its relation to conception

Group	Females	After estrus						
		Females menstruating within 5 days	Females conceiving	Females conceiving and menstruating		Females not conceiving	Females not conceiving but menstruating	
	no.	no.	no.	no.	%	no.	no.	%
Heifers (open)	100	100
Cows (open).....	100	61
Heifers (bred)	100	81	61	52	85.25	39	29	74.36
Cows (bred).....	100	61	72	50	69.44	28	11	39.29

Menstruation was observed in everyone of the 100 heifers not bred during estrus. In the group of 100 cows not bred during estrus, a total of 61 had a menstrual discharge. Of the lot of 100 heifers bred at estrus, a total of 81 menstruated. Sixty-one heifers conceived and of these, 52 or 85.25 per cent menstruated within five days while 9 or 14.75 per cent showed no evidence of menstruation. From the 39 heifers which did not conceive after breeding, 29 or 74.36 per cent menstruated while 10 or 25.64 per cent showed no signs of menstruation. In the group of 100 cows bred at estrus, 61 menstruated within five days. Seventy-two cows conceived and of these 50 or 69.44 per cent menstruated while 22 or 30.56 per cent did not men-

struate. Of the 28 cows which did not conceive after breeding, 11 or 39.29 per cent menstruated and 17 or 60.71 per cent did not menstruate.

The estimated amount of menstrual fluid and the time after estrus when this was discharged was recorded and these data are presented in table 2.

TABLE 2
Time of menstruation and amount of menstrual fluid

Group	Females grouped according to estimated quantity of menstrual fluid			Females grouped according to time of menstruation			
	Slight	Moderate	Profuse	Days after estrum			
				1st	2nd	3rd	4th
	no.	no.	no.	no.	no.	no.	no.
Heifers (open)	32	59	9	4	82	12	2
Heifers (bred—no conception)	11	15	3	1	16	8	4
Heifers (bred—conceived)	17	29	6	6	39	7	...
Cows (open)	22	31	8	8	49	4	...
Cows (bred—no conception)	4	7	5	6	...
Cows (bred—conceived)	22	28	...	8	34	8	...
Total	108	169	26	27	225	45	6

In the above table, the data indicate that for the 303 individuals that menstruated, 108 or 35.64 per cent had a slight amount, 169 or 55.78 per cent a moderate, and 26 or 8.58 per cent a profuse amount of menstrual fluid. The second part of table 2 in which the females were grouped according to time of menstruation in days after estrus shows that 27 or 8.91 per cent menstruated on the first day, 225 or 74.26 per cent on the second day, 45 or 14.85 per cent on the third, and 6 or 1.98 per cent on the fourth day following estrus.

DISCUSSION

This study offers evidence that the common belief among livestock men and veterinarians that a heifer or cow which menstruates a few days after service did not conceive is erroneous. These data show to what a surprising degree this supposition is in error. The data presented reveal that of the 100 heifers which were bred, 61 conceived and that 85.25 per cent of those conceiving also menstruated. A group of 100 cows bred resulted in 72 conceptions and 69.44 per cent of those conceiving also menstruated. It was found that the frequency of menstruation in heifers is greater than in older cows. This may be due to the fact that heifers as a rule are more excited at heat than are older cows which may result in the genital organs becoming more engorged with blood. The genital organs of the cow also are longer and extend further forward and occupy more of an abdominal position as compared to the pelvic position in heifers and this may have a tendency to prevent the flow of the menstrual fluid from the vulva of some

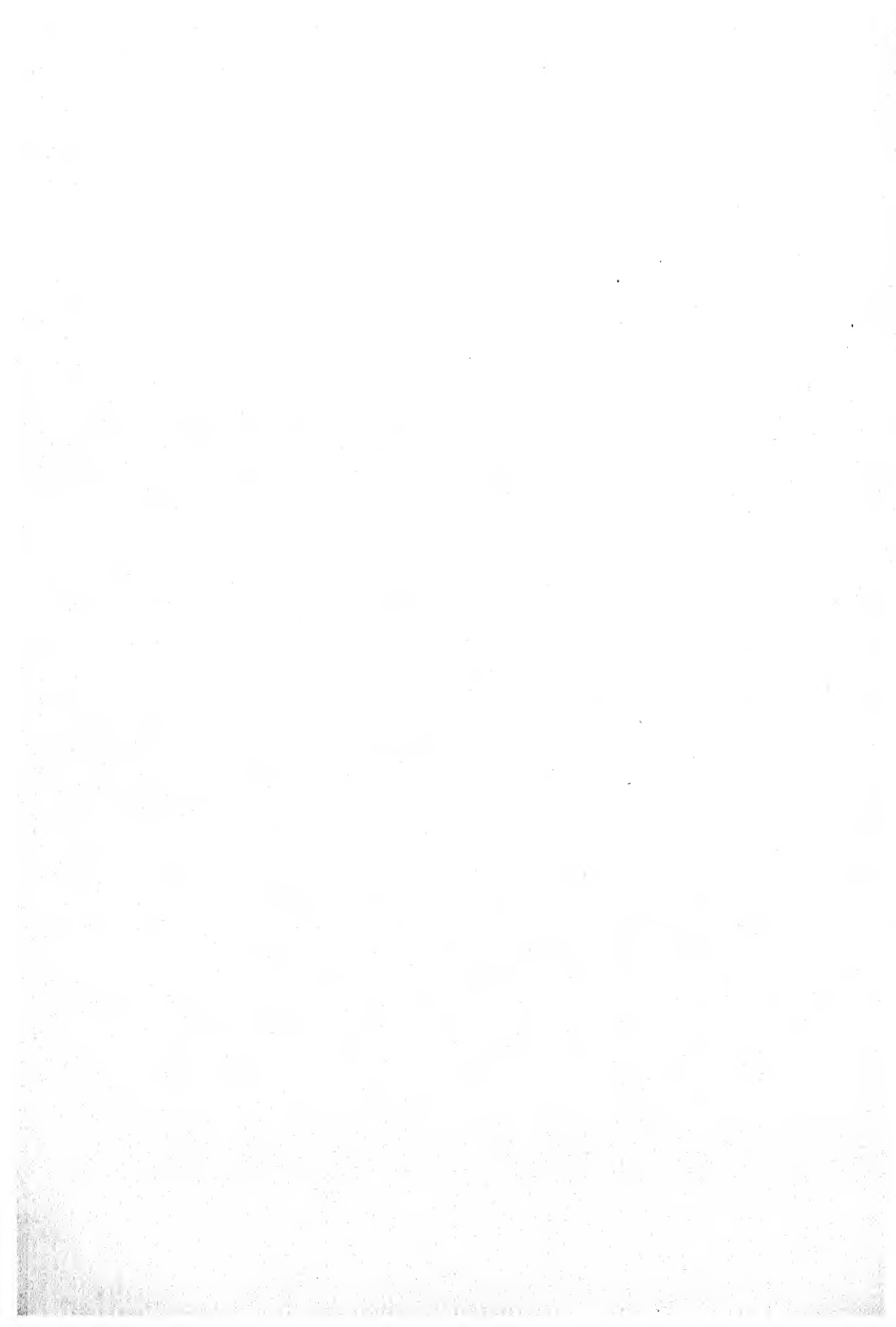
of the older animals. Another point that might be mentioned is that the amount of menstrual fluid is possibly dependent on the thickness and toughness of the endometrium. This will vary with age and number of calvings and there will be some variation between individuals. The data presented indicate that of the 303 females that menstruated, 225 or 74.26 per cent passed off the menstrual fluid on the second day following estrus.

SUMMARY

Observations as to the external evidence of the occurrence of menstruation were made at six different times daily on each of five days following estrus, for four lots of dairy females each consisting of 100 animals. One hundred heifers and an equal number of cows were observed after estrus, when breeding did not occur, and 100 heifers and 61 cows showed evidence of menstruation. Two similar groups of 100 heifers and 100 cows were bred during estrus. Of the 100 heifers bred at estrus, 81 menstruated. Sixty-one of the heifers conceived and 52 or 85.25 per cent of those conceiving also menstruated while of the 39 that did not conceive, 29 or 74.36 per cent menstruated. Of the 100 cows bred at estrus, 61 menstruated. Seventy-two of these cows conceived, and 50 or 69.44 per cent of those conceiving also menstruated, while of the 28 that did not conceive, 11 or 39.29 per cent menstruated. The data do not indicate any definite relationship between breeding and conception as affecting menstruation. When external evidences of menstruation were observed a total of 303 of the 400 individuals menstruated and 225 or 74.26 per cent showed this menstrual discharge on the second day following estrus.

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FINAL REPORT OF COMMITTEE ON METHODS OF DETERMINING THE CURD TENSION OF MILK

The curd tension of milk, while probably not wholly satisfactory as a measure of digestibility and suitability of milk for infant use, nevertheless has been tacitly accepted as an indication of these characteristics. The recent action of the Council on Foods of the A.M.A. in "accepting" homogenized milks with soft curd claims and establishing a limit of 20 grams tension for such milks has further added to the repute of the curd tension value. Some municipalities have set, or are contemplating establishing, curd tension standards for homogenized milk, the production of which is increasing rapidly. It seems particularly desirable, therefore, to publish the procedure for making curd tension determinations which was approved at the Thirty-sixth Annual Meeting of the American Dairy Science Association, June 1941.

DETERMINATION OF THE CURD TENSION OF MILK

Reagent

The coagulant consists of .08 N hydrochloric acid to which U.S.P. (1:3000) dry pepsin has been added at the rate of 450 mgms. per 100 ml. This solution may be kept under refrigeration and away from light for a period of not more than 10 days without losing its effectiveness but, in case of doubt, results obtained with it must be checked against results obtained using a freshly prepared coagulant.

Apparatus

The instrument used in measuring the curd tension shall indicate the grams of force* necessary to cut the coagulum, obtained as described below, using the standard type of knife. The motion of the knife or the vessel containing the coagulum shall be automatic and at the rate of one inch in 7 to 8 seconds, obtained by lowering the knife or by raising the vessel containing the coagulum. The sensitivity of the instrument shall be such that readings can be made to an accuracy of ± 1.0 gram.

Standard Knife

The knife used as a part of the instrument for measuring the curd tension shall consist of eight radial blades each having $\frac{3}{16}$ inches of lineal cutting edge and being .020 inches thick, spaced equally and enclosed by a circular or ring blade, $1\frac{3}{4}$ inches outside diameter, $\frac{3}{16}$ inches high and .031 inches thick. The radial blades shall be attached to the inside of the circular or ring blade and extend upward from it a distance of $2\frac{1}{16}$ inches,

* Submarine Signal Company Curd Tension Meters made prior to July 1941 do not read in grams. Readings may be converted to grams, however, by adding 10 per cent (multiplying by 1.10).

being reduced to a width of $5/32$ inches above the circular or ring blade. Their upper ends shall be curved inward and attached to a central spindle $\frac{15}{16}$ inches in diameter. The lower or cutting edge of the circular or ring blade shall be tapered from the outside at an angle of 30 degrees to the knife axis, to a dull knife edge. The lower or cutting edges of the radial blades shall be tapered, on each side, at an angle of 15 degrees to the knife axis, to a dull knife edge. The cutting edges of the ring blade and the radial blades shall lie approximately in the same plane, deviating not more than $1/32$ inches. The total linear cutting edge of the knife shall be 9.8 inches ± 0.10 inches. All joints shall be mortised and soldered smoothly and the knife constructed of a non-corrosive metal.

Coagulation Vessel

The receptacle used for coagulating the milk shall be a heavy walled jar 3 to 4 inches high (inside) and having an inside diameter of $2\frac{3}{4}$ inches $\pm \frac{1}{8}$ inch. The jars listed below have been found to meet these specifications satisfactorily and undoubtedly others will also be found which conform.

W. M. Welch Scientific Company, Chicago—Museum jar No. 4612A
(8 oz.)

Central Scientific Co., Chicago—Museum jar No. 10396 ($7\frac{1}{2}$ oz.)

Owens Illinois Glass Co., Toledo—Mayonnaise jar (8 oz.)

Sample Pipette

The pipette used for measuring and adding the milk sample to the jar containing the coagulant shall be one made to deliver 100 ml. which has had the tip removed and which empties water by gravity in approximately 4.5 seconds.

Water Bath

A suitable water bath shall be used to temper the jars containing the coagulant before the sample of milk is added and to hold the milk and coagulant at the proper temperature during the coagulating period. The water level shall come up on the outside of the jar to a point no lower than the milk and coagulant level on the inside of the jar. The volume of water shall be such that the temperature does not change more than 1.0° F. during the 10-minute period of coagulation and should preferably be agitated.

Procedure

Introduce 10 ml. of the coagulant into the coagulating vessel and set the vessel in the water bath, having previously adjusted the temperature of the water to 95° F. The vessel containing the coagulant should be tempered for no less than 3 minutes before introducing the milk sample.

The undiluted milk to be tested is tempered to 95° F. or 96° F. as experience dictates being careful not to exceed 100° F. in the process. The tempering should be accomplished in a five-minute period immediately preced-

ing the pipetting of the sample. Introduce 100 ml. of the sample into the coagulating vessel using the tipless 100 ml. pipette. This is to be accomplished by holding the pipette vertically over the center of the vessel and blowing the sample from the pipette as rapidly as possible. No further mixing of the vessel contents is to be employed and the vessel should not be disturbed in the water bath. Place a watch glass or other suitable covering over the vessel immediately.

The contents of the coagulating vessel are held at $95^{\circ}\text{ F.} \pm 1.0^{\circ}$ for 10 minutes, the time in the water bath being adjusted so that the cutting of the curd by the curd tension instrument occurs at the expiration of 10 minutes ± 30 seconds from the time the sample was placed in the coagulation vessel.

The curd tension reading is the maximum reading which is obtained at the moment the knife penetrates the surface of the coagulum. The test shall be made in duplicate or replicate and results to be acceptable must not deviate more than 5 per cent from the average. The average of the results so checking constitutes the curd tension of the sample.

Remarks

The curd tension value of milk should not be considered an absolute index of its digestibility nor of its suitability for infant feeding purposes. The values do, however, correlate in a general way with these properties and the determination appears to be the best available, simple method for the purpose.

The method is conventional and the procedure must be followed closely otherwise considerable variation in readings may result. One very important factor is that of securing *uniform* mixing of milk and coagulant with *promptness* so that the mixture will be quiescent before coagulation commences.

Another factor which has been shown to somewhat influence results is the speed of warming the milk samples to the coagulating temperature. Where the milk is warmed very rapidly the results are slightly lower than where slow warming is practiced.

Adjusting the pH of milk for the purpose of making curd tension determinations is not a part of this method. Results obtained after such adjustments cannot be considered normal.

The determination of the curd tension of milk as here described is recommended for use with undiluted milk only. Some types of soft curd milk on dilution give results which cannot be considered meaningful.

C. J. BABCOCK

L. A. CHAMBERS

C. C. FLORA

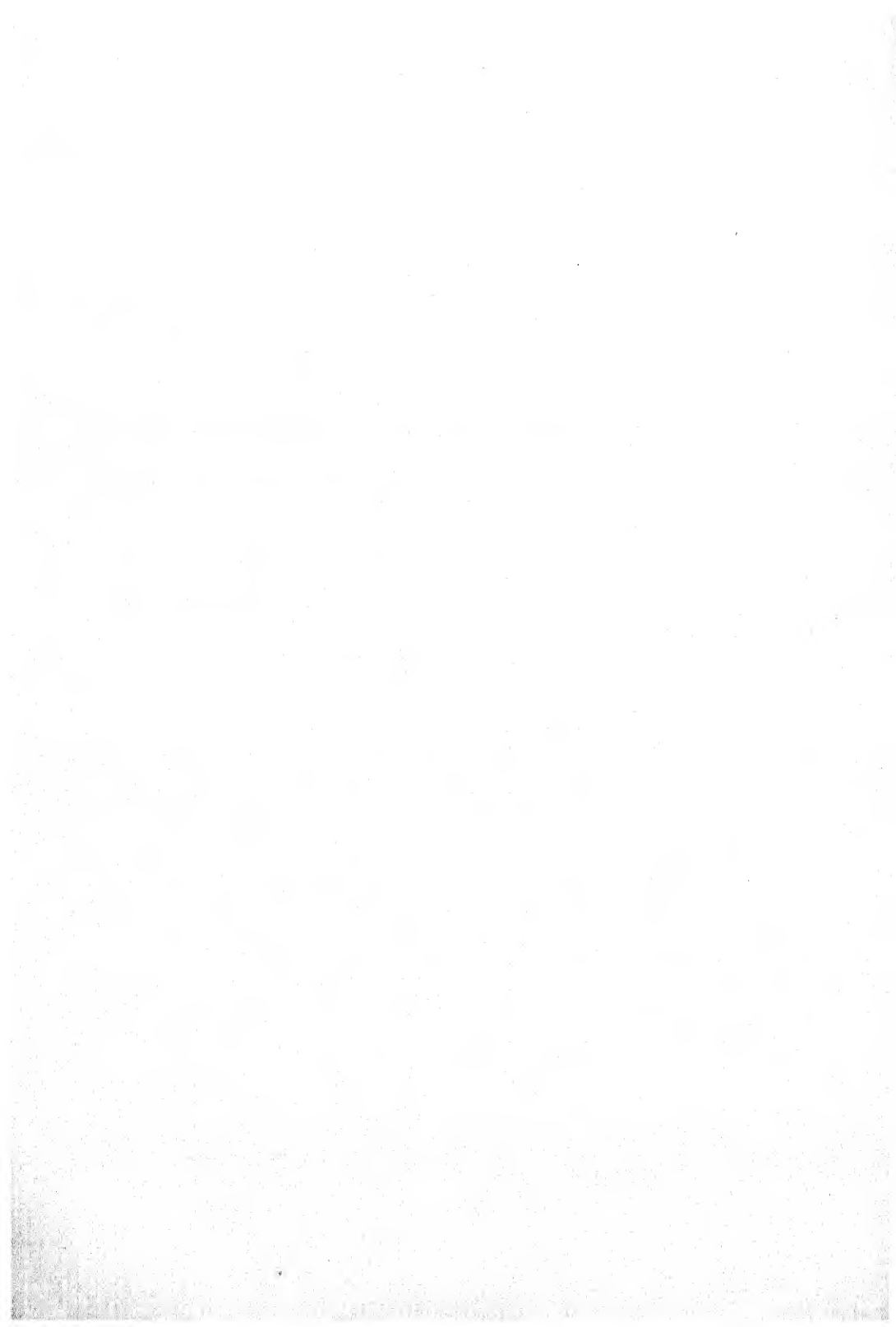
M. E. HULL

W. S. MUELLER

H. H. SOMMER

A. B. STORRS

F. J. DOAN, Chairman



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THE PRODUCTION AND USE OF CONCENTRATED SKIM MILK FOAM

B. H. WEBB

*Division of Dairy Research Laboratories, Bureau of Dairy Industry,
U. S. Department of Agriculture*

The foaming of skim milk during various manufacturing operations has long been a source of annoyance to dairy products manufacturers. Many reports deal with prevention and destruction of foam but apparently there have been few attempts to utilize the foaming property of skim milk. While fresh skim milk forms a relatively unstable surface foam, the results presented here will show that reconstituted dry skim milk or concentrated skim milk can often be whipped to a stiff white foam of high stability. Some effects of temperature and time of whipping, concentration of solids, and of some variations in manufacturing procedure upon skim milk foam production and stability are reported. A new food use for dry or concentrated skim milks which whip readily is indicated.

The foaming of milk has been studied by several investigators, a concise discussion being available in Fundamentals of Dairy Science (2). A recent study of Ansbacher, Flanagan, and Supplee (1) is concerned with the substances in milk which cause foam production. Sharp, Myers, and Guthrie (6) were unable to decrease the foaming capacity of skim milk by repeatedly foaming and removing the foam thus formed, nor did they find an accumulation of any major protein fraction in the foam. Studies on the effect of temperature upon the foaming of skim milk (3, 5) indicate that between the temperatures of 20° C. and 30° C. the foaming tendency is at a minimum. Foaming increases above and below this temperature range.

Evaporated milk can be whipped when it is maintained at low temperatures but since this product is manufactured from whole milk, its relatively high fat content inhibits the incorporation of a large quantity of air. Recently Leviton and Leighton (4) have discussed the depressing effect of fat upon skim milk foam.

EXPERIMENTAL

Foam was produced by whipping concentrated skim milk or reconstituted dry skim milk in a small electrically-operated mixer that maintained at high

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speed, 1,000 r.p.m. without a load. When skim milk powder was used, it was added to water in the mixer bowl and beaten at low speed for one minute before whipping at high speed. The time figures given with the data in this paper refer to the number of minutes the sample was whipped at high speed and do not include the one-minute mixing period. Most of the data were obtained by using reconstituted dry skim milks of 30 per cent solids. To prepare these milks 49- $\frac{1}{2}$ grams of dry skim milk were added to 110- $\frac{1}{2}$ cc. of water. An allowance of 3 per cent moisture was made for all dry milk samples. Different samples were whipped to secure the data for each time period. Percentage overrun was calculated as 100 times the difference between the weight of 175 cc. of milk measured before whipping and 175 cc. measured after whipping, divided by the weight of 175 cc. measured after whipping.

The stability of the foam produced by whipping was determined by observing the time required for the first drainage to appear in the bottom of a 160 cc. glass tumbler filled with the whipped milk. The limit of error in the whipping and stability tests was ± 5 per cent but the actual error in most cases was probably less than half of this figure.

Viscosity measurements were made on the foam drainage obtained from 3 to 6 hours after whipping. A McMichael viscosimeter was used and the wires were standardized with sugar solutions.

The temperature at which all whipping, drainage, and viscosity tests were conducted was 20° C. unless otherwise stated.

The dry skim milks used for the experiments reported in tables 1 and 2 were commercial products prepared for use in bread making. Fresh skim milk from the Beltsville Research Center was used to obtain the data of table 3. The eight samples of skim milk required for the experiments of table 4 and figure 1 were derived from various sources as indicated. On a basis of 9 per cent total solids all skim milks contained less than 0.01 per cent fat by the Babcock test. It was important to use milks of low fat content since fat retarded overrun development.

RESULTS

The data of tables 1 and 2 show that an increase in whipping tempera-

TABLE 1

The effect of temperature upon the whipping properties of dry skim milk reconstituted to 25 per cent solids

Whipping temperature	Overrun after whipping 3 min.	Temperature of room during tests	Stability of foam
°C.	%	°C.	min.
20	368	20	30
30	400	30	25
40	444	30	20
50	489	30	16
70	478	30	15

ture or a decrease in solids cause an increase in overrun but a decrease in foam stability.

TABLE 2

The effect of the concentration of solids upon the whipping properties of reconstituted dry skim milk

Solids content of mix	Overrun after whipping 3 min.	Stability of foam
%	%	min.
10	412	1½
15	422	5½
20	402	11
25	331	21
30	307	47

The experiments reported in table 3 show the improvement in whipping properties brought about by the high heat treatment of one skim milk while more detailed data on whipping and related tests with 9 dry skim milks are presented in table 4. Whipping data for 8 of these milks are plotted in figure 1.

TABLE 3

The effect of the heat treatment of a skim milk upon the whipping properties of its condensed or reconstituted dry product. All milks contained 30 per cent solids when whipped

No.	Treatment of skim milk sample	Overrun after whipping 3 min.	Stability of foam
		per cent	min.
1	Forewarmed at 65° C. 15 min., condensed to 30 per cent solids	106	0
2	Forewarmed at 95° C. 15 min., condensed to 30 per cent solids	240	7
3	Same as No. 2 but superheated to 100° C. after condensing	259	18
4	Same as No. 2, held cold overnight, heated to 50° C., spray dried	252	> 45

The results reported above were made the basis for experiments on some new food uses for skim milk. High foaming skim milks were whipped to stiffness at concentrations of 25 and 30 per cent solids and when sweetened, used as topping for beverages such as hot chocolate. These whips were used as a base for home frozen ice cream after the addition of flavoring and other fat-free ingredients. For food products requiring a permanent retention of air it was necessary to increase the stability of the skim milk foam. This was done by bringing about a mild coagulation of the casein after development of the whip, thus setting the foam structure. The use of heat, rennet, and acid as stabilizing agents was investigated. Attempts were made to start an incipient coagulation of the casein by heating the reconstituted milks over

TABLE 4

*Whipping properties and heat stability of samples of reconstituted dry skim milk.
The process used in the manufacture of these milks is given below fig. 1*

No.	Overrun after whipping:			Stability of foam			Viscosity of drainage	Time of coagulation at 125° C.
	2 min.	5 min.	10 min.	2 min. whip	5 min. whip	10 min. whip		
	per cent	per cent	per cent	min.	min.	min.	centipoises	min.
1	371	436	484	55	56	54	43	17
2	310	400	443	107	120	90	87	60
2S	257	370	411	57	74	67
3	217	353	413	4	21	29	20	156
4	188	294	367	4	9	17	31	160
5	167	244	258	19	28	35	148	37
6	88	154	245	8	18	41	97
7	80	130	214	> 24 hours			15
8	29	93	180	> 24 hours			< 1

The whipped samples contained 30 per cent milk solids, the heat-coagulated milks contained 9 per cent solids, and sample No. 2S was No. 2 milk with 25 per cent milk solids, 15 per cent sugar, and 60 per cent water.

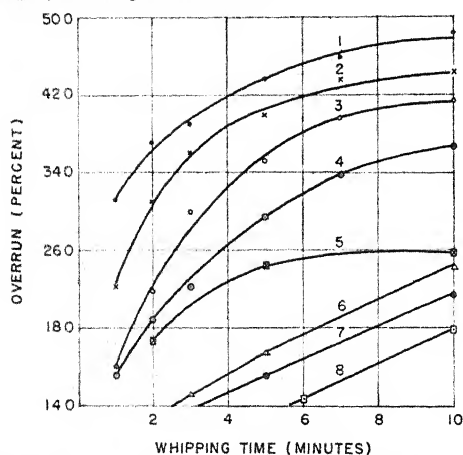


FIG. 1. Showing the development of overrun in dry skim milks reconstituted to 30 per cent solids and whipped for different periods of time. The dry skim milks were made by the methods indicated:

1. Vacuum drum dried commercial sample.
2. Spray dried commercial sample prepared for baking.
3. Fresh skim milk, forewarmed at 65° C., 20 minutes, condensed under vacuum to 30 per cent solids and spray dried.
4. Same milk and treatment as No. 3 but not condensed before drying.
5. Spray-dried commercial sample prepared for baking.
6. Same milk as No. 3, forewarmed at 95° C. for 20 minutes, spray dried.
7. Same milk and treatment as No. 3, but the condensed milk was superheated over a steam bath to 90-96° C. for 10 minutes and dried at once. Total time from start of superheating to end of drying operation was 45 minutes.
8. Atmospheric roll-dried commercial sample.

a steam bath while they were being whipped. The time required for the development of a coagulum was so long that the flavor was adversely affected and the method was not suited for stabilizing whips. The foam could be stabilized well with rennet when the factors controlling rennet coagulation were standardized, but this required exact laboratory methods. Nevertheless, a sweetened, rennet-stabilized, skim milk whip produced an attractive "Junket" type of desert when properly prepared. The foam was easily stabilized by the addition of acid. Pulped fruit from prunes, apricots, or berries was used as a source of acid. The product thus obtained made an excellent fruit whip which was equal to egg white fruit whips in flavor and often superior to them in stability. When pulp such as that of the prune was present, the whip was stable for many hours. Suitable formulas for fruit whips were developed. That for prune whip follows:

Dry skim milk	48 gr.
Powdered sugar	28 gr.
Water ($\frac{1}{2}$ cup)	113 gr.
Prune pulp	150-200 gr.

The skim milk and sugar were added to the water in the bowl of an electric mixer, the preparation whipped for at least 5 minutes and the prune pulp then quickly mixed in at low speed. Excessive mixing or stirring after the addition of acid foods caused some wheying off. Whipped mixtures of this type were frozen without stirring to produce smooth textured frozen desserts.

Fruit pulp was found to be necessary for the adequate stabilization of an acidified skim milk whip. Whips made with lemon juice wheyed off quickly, but when banana pulp and lemon juice were used, greater stability was attained. Excessive quantities of acid produced a whip with curd particles of objectionable size.

A milk powder flavor was sometimes noticeable in the finished whips. Fresh powder of good flavor was necessary for the production of an attractively flavored whip.

DISCUSSION

Foam of high air content and good stability may be secured by whipping properly heat-treated skim milks of 25 to 30 per cent solids content for several minutes. The data, however, are not complete enough to establish manufacturing procedures which will produce uniformly high whipping skim milks. The behavior of milks No. 3, No. 4, No. 6, and No. 7 (table 4 and fig. 1) indicates that increasingly severe heat treatment finally causes a decrease in whipping properties which is accompanied by a lowering in the heat stability of the protein. Milk dried on an atmospheric drum drier (No. 8) is subjected to intense heat when it is in a highly concentrated condition just before all the moisture is evaporated. The result of this drastic treatment is a powder of poor whipping properties and low heat stability.

It is probable that an optimum heat treatment which produces maximum whipping properties exists for each milk and that identical heat treatment does not necessarily produce the same whipping properties in different milks. Milk No. 4, table 3, developed 252 per cent overrun, while a milk obtained from the same source several weeks later and treated in the same way whipped to 367 per cent overrun with a stability of 83 minutes.

Commercial samples of dry skim milk prepared especially for baking whipped well, but some developed greater overrun than others. The preparation of a dry skim milk with high whipping properties should evidently involve high heat treatment somewhat similar to the procedure used in the manufacture of powder for baking purposes.

Particle size of insoluble roller dried powder exerts some influence upon air incorporation during whipping. An overrun of 156 per cent was produced after whipping a commercial powder of this type for 10 minutes. After the product was ground in a ball mill for 2 and 6 hours and whipped under the same conditions, overruns of 200 per cent and 251 per cent, respectively, were obtained.

SUMMARY

1. Reconstituted dry skim milks and condensed skim milks of 25 per cent to 30 per cent solids content were mechanically whipped in a few minutes to a stiff white foam. An overrun of 150 to 450 per cent and a foam stability of 10 to 90 minutes were found for different milks.

2. Wide variations were observed in the whipping properties of skim milks. High heat treatment usually improved whipping properties. Commercial milks prepared for baking purposes generally exhibited excellent whipping ability.

3. Skim-milk-whips were set by rennet or acid, but subsequent disturbance caused wheying off. Fruit-whips similar to an egg white product were prepared by adding sugar to the skim milk foam and stabilizing the whip by stirring fruit pulp into it.

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THE UTILIZATION OF UREA BY RUMINANTS AS INFLUENCED BY THE LEVEL OF PROTEIN IN THE RATION

M. I. WEGNER, A. N. BOOTH, G. BOHSTEDT, AND E. B. HART

Departments of Biochemistry and Animal Husbandry, University of Wisconsin, Madison

INTRODUCTION

The ability of ruminants to utilize simple nitrogen compounds such as urea or ammonium bicarbonate as a source of protein has been explained by bacterial synthetic activity occurring in the rumen and reticulum of these animals (1). A large and varied microflora is known to exist in the rumen and to account for this action. The media in which these microorganisms grow is determined by the ration fed. Since the kind and number of microorganisms are probably influenced by the composition of the medium, the possibility exists that by varying the ration fed, a change of the flora in the rumen could be produced and thereby a change in the synthetic reactions. In a previous "in vitro" experiment (2) it was found that the level of protein in a medium influenced the rate and amount of conversion of the ammonia (urea) when this medium was inoculated with microorganisms from the cow's rumen. As the protein level was increased from 2.5 grams to 5 grams of casein per 100 cc. of medium, the conversion of the added ammonia became negligible. The question arises as to whether this same phenomenon would occur in the rumen of animals fed urea. Acquisition of such knowledge would be important, not only from an academic viewpoint but also from a practical and economic one, since simple nitrogen compounds can be used as protein substitutes in rations of ruminants. With these facts in mind an experiment was set up to determine the effect of the level of protein fed on urea nitrogen utilization in the rumen.

EXPERIMENTAL

The data presented in this paper were obtained through the use of a 1000 pound Holstein heifer with a rumen fistula equipped with a removable rubber plug to facilitate sampling. The animal was fed twice daily at 8 A.M and 8 P.M. The daily ration consisted of corn silage 15 pounds, timothy hay 4 pounds, and a basal grain mixture of 4 pounds. The basal grain mixture was made up of ground yellow corn 50 per cent plus ground oats 50 per cent. The only variable in all the experiments was the grain mixture. These variations are listed in table 1. The heifer maintained its weight on

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the various rations fed throughout the experiment. Since the ration was always completely consumed within one hour after feeding, sampling was

TABLE 1
Variation of protein ($N \times 6.25$) content of the grain mixture

	Protein in basal con- centrate theory	% protein increase due to L.O.M. added	% protein equiv. in- crease due to added urea	Total N as protein in concentrate	
				Theoretical	Actual
Experiment I—Variation of protein and urea levels					
Trial 1	12.0	0	0	12.0	11.3
2	12.0	0	6	18.0	17.1
3	12.0	4	2	18.0	17.1
4	12.0	8	4	24.0	23.3
5	12.0	12	6	30.0	31.1
Experiment II—Variation of protein level with L.O.M.—urea constant					
Trial 1	12.0	0	0	12.0	11.3
2	12.0	0	6	18.0	17.1
3	12.0	6	6	24.0	23.5
4	12.0	12	6	30.0	31.1
Experiment III—Variation of level of urea added—protein constant					
Trial 1	12.0	0	0	12.0	11.3
2	12.0	0	6	18.0	17.1
3	12.0	0	12	24.0	24.0
4	12.0	0	18	30.0	31.1

initiated at that time and continued throughout the day until the ammonia had returned to the basal level. All trials were repeated on alternate days at least three times before passing on to the next experiment. Table 2 contains the data obtained from three runs of the same trial as an example of the uniformity of results.

The determinations made on each rumen sample were ammonia, non-protein nitrogen (tungstic acid non-precipitable nitrogen), total nitrogen, and dry matter. The methods used in the sampling and in the determinations have been reported in a previous publication (3). With every ration tried it was found that the added urea-nitrogen was always hydrolyzed to ammonia within one hour after feeding. All results are calculated on a dry matter basis. When changing from one ration to another (change of the grain mixture composition) the animal was allowed to equilibrate for a week or more before sampling was started.

Utilization of the urea added was studied from three standpoints: first, varying both the oil meal and the urea added to the grain mixture; secondly, keeping the urea added constant and varying the oil meal; and thirdly, keeping the oil meal constant and varying the urea. In this manner the effect of both variables—urea and oil meal—on conversion could be studied. The results obtained are given in the accompanying charts.

DISCUSSION

In experiment I the concentrates consisted of mixtures having a protein content comparable to those sold commercially. Therefore it was desirable to know if urea would be utilized when these types of concentrates containing urea were fed with corn silage and timothy hay, a practical dairy ration. In figure 4 it is seen that all of the ammonia (urea) disappears at the end of six hours in all the trials but with one exception, namely, trial 5. In this trial the protein level of the grain mixture was 24 per cent and the urea added equivalent to 6 per cent of protein—a total of 30 per cent protein equivalent. This protein level in the grain mixture is one sometimes used, but is unnecessarily high. If the corresponding curves representing the total nitrogen

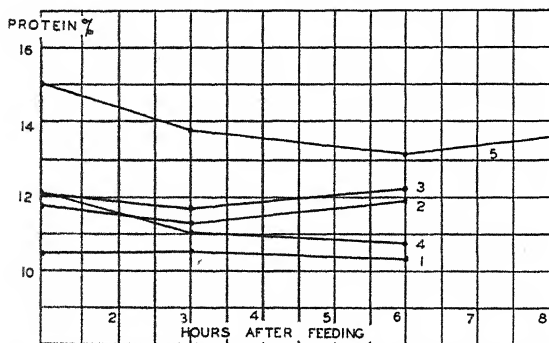


FIG. 1. Exp. I—Protein ($N \times 6.25$) in rumen contents (dry basis).

1. Protein in Conc. 11.3%
2. " " " " + 6% protein equiv. as urea.
3. " " " " + 4% oil meal protein + 2% of protein equiv. as urea.
4. " " " " + 8% oil meal protein + 4% of protein equiv. as urea.
5. " " " " + 12% of oil meal protein + 6% of protein equiv. as urea.

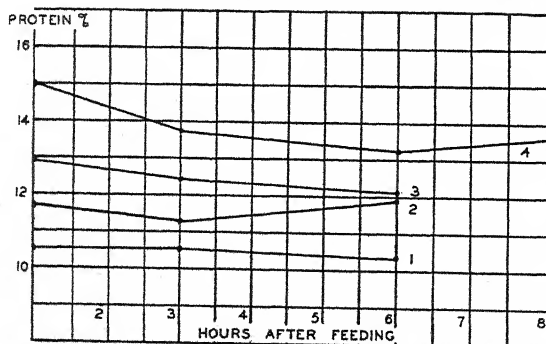


FIG. 2. Exp. II—Protein ($N \times 6.25$) in rumen contents (dry basis).

1. Protein in Conc. 11.3%
2. " " " " + 6% protein equiv. as urea.
3. " " " " + 6% oil meal protein + 6% protein equiv. as urea.
4. " " " " + 12% oil meal protein + 6% protein equiv. as urea.

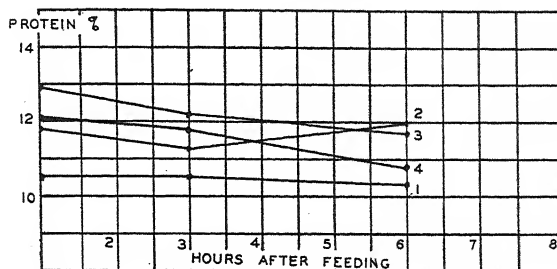


FIG. 3. Exp. III—Protein ($N \times 6.25$) in rumen contents (dry basis).

1. Protein in Conc. 11.3%
2. " " " " + 6% protein equiv. as urea.
3. " " " " + 12% " " " "
4. " " " " + 18% " " " "

content of the rumen ingesta are examined (fig. 1) it is seen that here all the curves, again with one exception, lie quite close together (trial 5). In other words, excessively high protein levels found in the rumen will influence the rate of disappearance of ammonia. This verifies the results previously reported in "in vitro" experiments (2), where it was found that as the

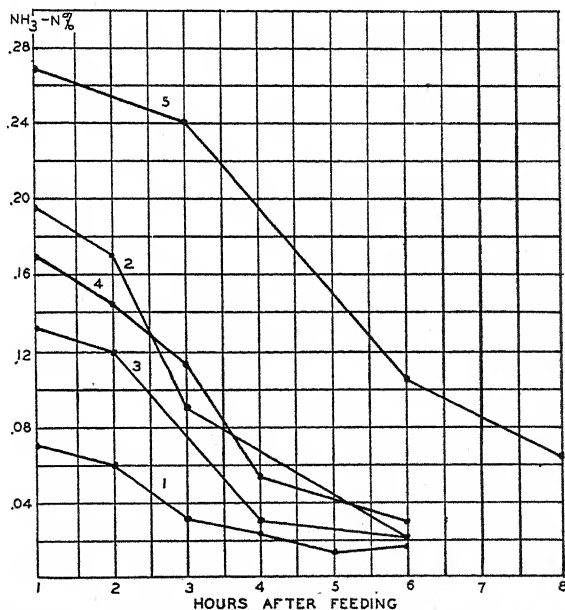


FIG. 4. Exp. I— NH_3-N in rumen contents (dry basis).

1. Protein in Conc. 11.3%
2. " " " " + 6% protein equiv. as urea.
3. " " " " + 4% oil meal protein + 2% of protein equiv. as urea.
4. " " " " + 8% oil meal protein + 4% of protein equiv. as urea.
5. " " " " + 12% of oil meal protein + 6% of protein equiv. as urea.

protein (casein) in the medium was increased above 2.5 grams per 100 cc., the rate of conversion of inorganic nitrogen to protein was decreased. Judging from the results in experiment I, utilization of urea nitrogen will take place with protein concentrate levels as high as found in trial 4 (basal grain mixture plus oil meal to 20 per cent protein plus urea to 24 per cent protein equivalent) other parts of the ration being similar.

Examination of the results in experiment II (figs. 2 and 5, in which the urea added to the grain mixture is constant—6 per cent protein equivalent—and the protein content varied by adding oil meal) again illustrates the point that as the total nitrogen level in the rumen is increased above 12 per cent expressed as protein (fig. 2, trials 3 and 4) by feeding high protein concentrates (trial 3—18 per cent protein plus urea equivalent to 6 per cent protein; trial 4—24 per cent protein plus urea equivalent to 6 per cent protein) the utilization of ammonia is retarded (fig. 5, trials 3 and 4). Under the conditions of this experiment the utilization of urea fed at a level of 2.5 per cent in grain mixture decreased when the protein level of the concentrate was greater than 18 per cent.

When the low protein concentrate (grain mixture) equivalent to 11.3

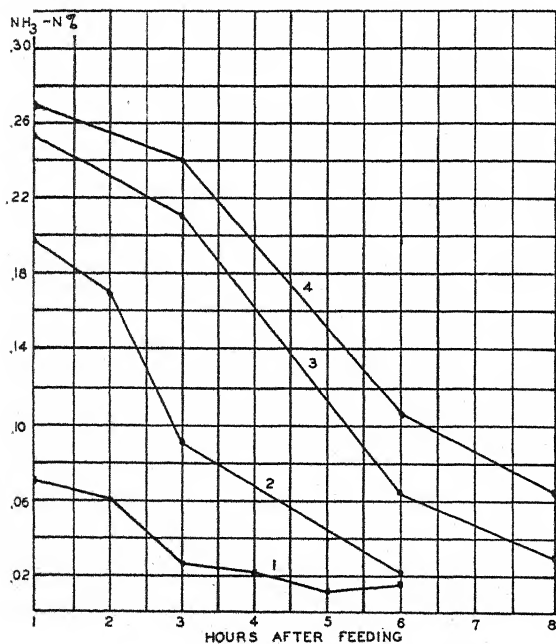


FIG. 5. Exp. II—NH₃-N in rumen contents (dry basis).

1. Protein in Conc. 11.3%
2. " " " " + 6% protein equiv. as urea.
3. " " " " + 6% oil meal protein + 6% protein equiv. as urea.
4. " " " " + 12% oil meal protein + 6% protein equiv. as urea.

per cent protein was fed as in experiment III it was found that the urea added to the concentrate could be raised to a 4.5 per cent level (12 per cent protein equivalent) before there was a pronounced delay in the disappearance of the ammonia from the rumen (fig. 6, trials 3 and 4). It will also be noticed in figure 3 that the protein content of the rumen ingesta remained near the basal level. When large amounts of urea were added to this low protein concentrate as in trial 3 (12 per cent protein equivalent) and trial 4 (18 per cent protein equivalent) the rate of disappearance of the ammonia from the rumen was much faster (fig. 6) than when urea was added to the high protein concentrates as were fed in experiments I and II (figs. 4 and 5).

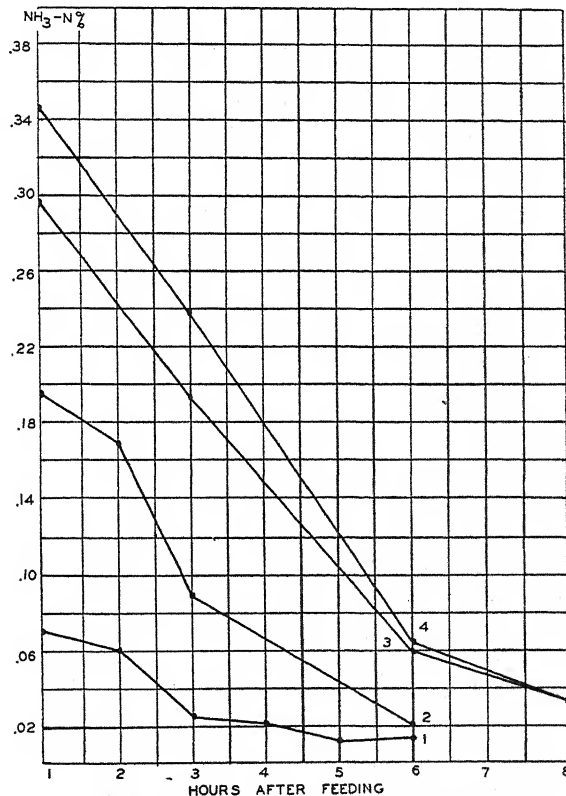


FIG. 6. Exp. III—NH₃-N rumen contents (dry basis).

1. Protein in Conc. 11.3%
2. " " " " + 6% protein equiv. as urea.
3. " " " " + 12% " " " "
4. " " " " + 18% " " " "

Figures 7, 8 and 9 contain the values obtained from determinations of non-protein nitrogen on several of the trials in each experiment. On

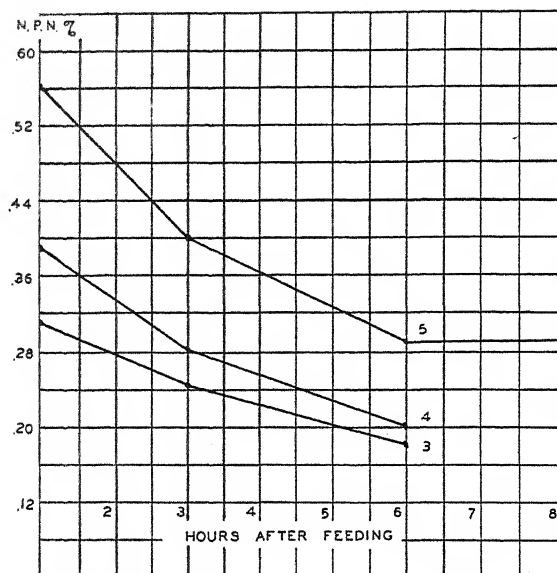


FIG. 7. Exp. I—N.P.N. rumen contents (dry basis).

3. Protein in Cone. 11.3% + 4% oil meal protein + 2% of protein equiv. as urea.
 4. " " " " + 8% oil meal protein + 4% of protein equiv. as urea.
 5. " " " " + 12% of oil meal protein + 6% of protein equiv. as urea.

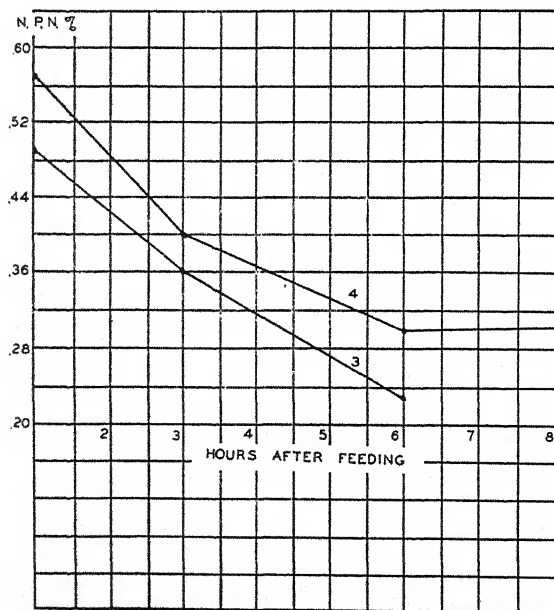


FIG. 8. Exp. II—N.P.N. rumen contents (dry basis).

3. Protein in Cone. 11.3% + 6% oil meal protein + 6% protein equiv. as urea.
 4. " " " " + 12% oil meal protein + 6% protein equiv. as urea.

examination it is seen that variations in these curves are directly related to the ammonia variation, since the ammonia is included in the non-protein nitrogen. When the variation in ammonia content is taken into consideration these curves become quite uniform.

The question may be raised as to whether the disappearance of ammonia is due to a conversion to protein by the microorganisms or simply a passage from the rumen unchanged. Some of the inorganic nitrogen may leave the rumen without being converted to protein. However, in view of the present experiments where it was possible to show a decided delay beyond 6 hours in the decrease of ammonia by a high protein content of the rumen ingesta

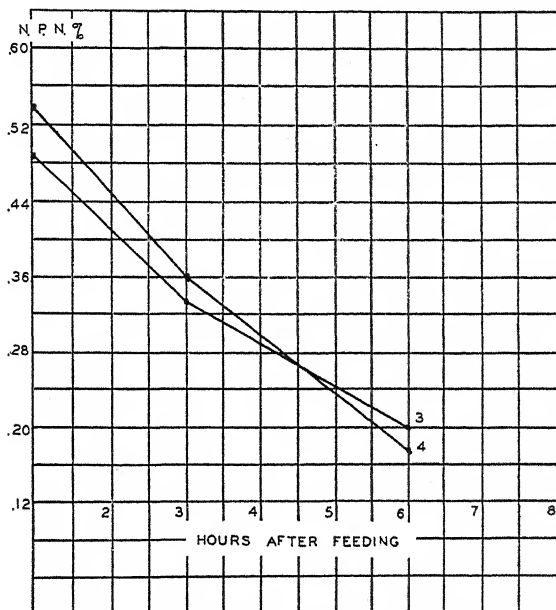


FIG. 9. Exp. III—N.P.N. rumen contents (dry basis).

3. Protein in Conc. 11.3% + 12% protein equiv. as urea.

4. " " " " + 18% " " " "

it appears that the disappearance taking place must also be due to a conversion to protein. Also, evidence of protein formation from urea in the rumen has already been reported in a previous publication (3).

Another factor which might easily influence the rate and extent of conversion of ammonia to protein in the rumen is the available carbohydrate fed. Experiments are now in progress to determine how the amount and kind of carbohydrate and the length of time it remains in the rumen will affect the rate of utilization of ammonia.

SUMMARY

1. The protein content of rumen ingesta showed a decided increase when the level of protein in the concentrate fed was increased to 24 per cent.

2. The rate of conversion of urea nitrogen to protein in the rumen decreased as the protein level of the rumen ingesta became greater than 12 per cent.

3. When the level of protein in the concentrate fed was increased to more than 18 per cent the rate and extent of conversion of added urea nitrogen to protein began to decrease.

4. When no linseed oil meal was added to the basal grain mixture (11.3 per cent protein), the added urea was utilized up to a level of 4.5 per cent (protein equivalent of 12 per cent) of the grain mixture.

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EFFECT OF STILBESTROL ON THE MAMMARY GLAND OF THE MOUSE, RAT, RABBIT, AND GOAT*

A. A. LEWIS AND C. W. TURNER

Department of Dairy Husbandry, University of Missouri, Columbia, Missouri

If knowledge of the hormonal causes of the growth and lactation of the mammary gland is to be put to practical use it will be necessary to find cheap sources of the hormones and devise simple and effective methods of administration. This report is concerned with a first step in that direction. Work in this laboratory has shown that the estrogenic hormones stimulate growth of the mammary duct system by increasing the rate of secretion of mammodgen by the pituitary (1).

The recent synthesis of a compound called stilbestrol, which has very great estrogen-like physiological activity and still is relatively inexpensive in comparison to the natural estrogens, has stimulated considerable research. Stilbestrol has the further advantages that it is quite effective orally and when formed into pellets is slowly absorbed for months when implanted under the skin (2, 3, 4, 5). This chemical, which simulates the estrogens in its physiological properties, is produced at 1/50 the cost for equal activity. It is sufficiently low in cost at active dosages to consider its practical application in livestock therapy. With this in mind the authors are conducting experiments on the influence of stilbestrol on the mammary gland and milk secretion in laboratory and farm animals.

Many stilbestrol experiments of clinical nature or having no application to mammary development cannot be reviewed here (for these see ref. 6). Assays of the effect of stilbestrol on the genital organs show that one-half gamma produced estrus in 70 per cent of 175 gram spayed rats (7). Stilbestrol was found to be equal to, or five times as active as, estrone in producing vaginal cornification in rats and mice (4, 7, 8). Orally administered stilbestrol was twenty times as active in mice as estrone and 16 times as active as estradiol (7).

Van Heuverswyn *et al.* (9) found that groups of five male mice given 0.05, 0.2, and 2.0 mg. of stilbestrol, respectively, gave plus two, plus three and plus one rating of mammary duct responses after eight injections in 16 days.

Stilbestrol was found to be about one-fifth as active as estrone in causing proliferation of the mammary gland of spayed rats (10), and of guinea pigs (11).

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In contrast to these results, experiments conducted by the present authors showed stilbestrol to be more active than estradiol benzoate in causing mammary duct proliferation in male mice (12).

Mammary development in the human male from oral administration of stilbestrol has been reported (13). A total of 480 mg. were given over 96 days. Hard, firm masses 6 cm. in diameter and 2.5 cm. thick were palpable under the nipples. These masses were harder, and denser than result from the action of the natural estrogens. Similar results, although less development, were obtained in a six-year-old girl. Breast hypertrophy and genital changes were reported (14) in a castrate woman given 20.25 mg. of stilbestrol in 18 days followed by 30 rabbit units of progesterone in six days. MacBryde *et al.* (15) reported similar results with stilbestrol administered orally or by injection.

Proliferation of the mammary epithelium was revealed by biopsy specimens obtained before and after oral administration of 280 mg. of stilbestrol to a castrate woman. One milligram a day given orally for 14 days to a second woman caused painful breast swelling (16).

De Fremery (17) reported in 1936 that incision of the udders of virgin female goats with estradiol benzoate caused mammary growth. Initially slow udder development increased abruptly with changes of parturient type. Lactogen then produced abundant normal lactation.

Folley *et al.* (18) reported that stilbestrol dipropionate in oil applied to the udders of three virgin female goats, plus daily milking, caused the production of a maximum of 1500 cc. of normal milk daily. A normal lactation curve resulted. A virgin heifer similarly treated responded with colostrum secretion alone. Attempts to induce lactation with stilbestrol in male goats failed even when progesterone was added to the treatment.

Lewis and Turner (19) reported that daily subcutaneous injections of stilbestrol caused the initiation of lactation with a maximum production of about half the normal quantity of milk from two yearling goats during lactation periods of six months. A castrate and a normal kid lactated for 95 days. The effect of stilbestrol injections on two goats already in milk was not good as far as production was concerned.

PROCEDURE

Stilbestrol (4:4 dihydroxy α β diethyl-stilbestrol) was obtained in powder form from E. R. Squibb and Sons. For injection it was dissolved in ether, added to the oil carrier and the ether removed before a fan. The daily dosage was administered in 0.05 to 0.2 cc. of oil to mice and rats and 0.1 to 0.4 cc. to rabbits. Two milligram tablets of stilbestrol, from the Geo. A. Breon Co., Kansas City, were put in suspension in the drinking water for oral administration to mice and renewed daily.

The mammary glands were removed in toto at biopsy or necropsy, fixed

in Bouin's fluid and stained in Mayer's hematoxylin. After removal of the panculus carnosus muscle and overlying connective tissue, the glands of the rabbits were measured and the greatest diameter recorded. Rabbit glands were removed at about 20-day intervals. Pituitary lactogenic hormone was prepared by the Bergman-Turner (20) method in this laboratory from cattle anterior pituitary and was assayed by the McShan-Turner pigeon method (21). One international unit equals 0.8 McShan-Turner units. Three hundred and forty international units (approximately 11 i.u. per 100 gm. body weight) are required to produce lactation in normal pseudo-pregnant rabbits and constitute one rabbit unit (22). It was given to rabbits in 6 equal daily subcutaneous doses. The mammary glands were examined and a gland removed on the seventh day.

Goats were injected subcutaneously in the crops with stilbestrol in 0.2-0.5 cc. of oil daily. Half of the udder was removed under local anesthetic, frozen, cross sectioned by hand, fixed in Bouin's fluid, stained and mounted in isobutyl methacrylate polymer. Thinner, small sections were mounted on slides in clarite. Paraffin sections were also made.

The mice were fed a mixed grain ration containing cod liver oil plus Purina dog pellets. The rabbits were fed a mixed grain ration plus alfalfa hay. Goats were stabled continually and fed a mixed dairy ration plus alfalfa hay.

EXPERIMENTAL RESULTS

Mice. Groups of three to five male mice injected with stilbestrol daily for 14, 21, and 27 days responded with extensive development of the mammary duct system (table 1). Dosages of 0.167 to 0.5 gamma per day caused the production of glands extending 0.5 to 1.5 cm. in length, usually with fewer main ducts than in glands of normal female mice. In most cases early interlobular duct development was evident. In one case isolated clumps of secreting alveoli were found, as reported by Gomez and Turner (23) following anol treatment (fig. 1). This condition has been shown in this laboratory (unpublished) to result from estrone injection. In no case were lobules of alveoli well developed. There was considerable variation in development of glands from the same mouse, but large glands were found in each case. There was progressive development in the groups treated for 14, 21, and 27 days.

Groups of four spayed virgin female mice given 0.167, 0.5 and 1.5 gamma of stilbestrol per day for 11 to 21 days gave a varying response. In some cases only end-buds and duct growth were apparent, in others interlobular ducts had developed. A number had mammary elements expanded with secretion. Two showed small, isolated clumps of alveoli (fig. 1).

Stilbestrol has been reported to be comparatively much more active orally than the natural estrogens (8). Oral administration would appear to be of considerable advantage in practical application of hormone therapy

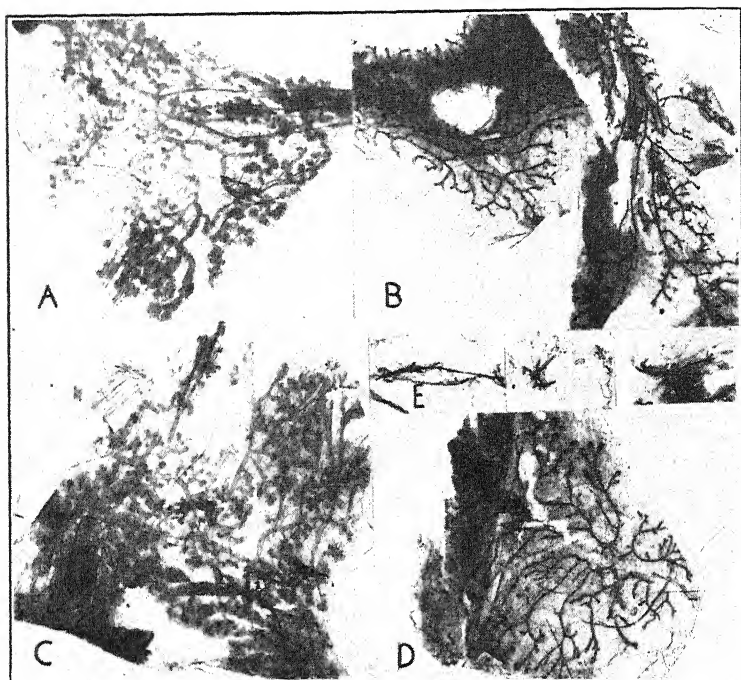


FIG. 1. a. Mammary gland from a spayed virgin mouse after daily injection of $1/6 \gamma$ of stilbestrol for 20 days. The ducts were expanded with secretion as were clumps of what appear to be alveoli. $\times 4.8$.

b. Control mammary glands from virgin female mice. Notice the smooth, unexpanded ducts. $\times 1.5$.

c. Mammary gland from a male albino mouse after 21 days treatment with $1/6 \gamma$ of stilbestrol daily. This gland was developed similarly to those of the female mouse shown in (a). Only one such case was found among a large number of males treated with stilbestrol. $\times 4.8$.

d. Typical mammary gland from a male mouse treated with stilbestrol for 14 to 27 days. Note active hyperplasia of ducts shown by enlarged, deeply staining end-buds. Some of these mice had ducts made rough in appearance by the beginning of interlobular duct development. This gland was similar to control glands in (e) before treatment. $\times 4.8$.

e. Three typical control mammary rudiments from male mice. $\times 4.6$.

to livestock. Stilbestrol administered in the drinking water to male mice caused extensive duct proliferation similar to that caused by injection. Groups of three and four mice were treated for 14 and 21 days. One-tenth to 0.4 gamma per day gave little or no stimulation while 0.5 to 1.5 gamma per day gave extensive proliferation (table 1).

From these results it is seen that stilbestrol readily caused extensive duct development in male mice either by injection or when administered in the drinking water. Stilbestrol carried the stage of gland development

TABLE 1
Effect of stilbestrol on the mammary gland of the mouse

Mode of administration	Number of mice	Days treated	Dosage, gamma per day	Results
Subcutaneous injection, males	5	14	1/6	1 neg. 4 pos. Largest gland in each case 0.6 to 0.9 cm.
	5	21	1/6	5 pos. Most glands 0.5 to 1 cm. diameter. One mouse had isolated alveolar clumps.
	4	27	1/6	Most glands 0.7 to 1.6 cm. extent. Roughened ducts.
Subcutaneous injection, males	5	14	0.5	Glands +1 to .9 cm. Largest .5 to .9 cm. in each case.
	5	21	0.5	Most glands .4 to .9 cm. extent. Roughened ducts.
	5	27	0.5	1 barely positive. Others, most glands 0.5 to 2 cm. extent. Roughened ducts.
Oral, in drinking water	3	14	0.1	One with teats had .4 to .6 cm. glands. Others negative.
	3	21	0.1	Negative.
			0.2 last 7 days	
	3	14	0.2	One with +1 gland. Others negative.
	3	21	0.2	Negative.
			0.4 last 7 days	
	3	14	0.4	One with a +1 gland. Others negative.
	3	21	0.4	2 strongly developed with .5 to .9 cm. ducts.
			0.8 last 7 days	
Males	3	14	0.5	Largest glands 0.6 to .8 cm.
	4	21	0.5	Most glands 0.4 to 1.2 cm. ducts.
	4	14	1.0	0.5 to 1.1 cm. roughened ducts.
	4	21	1.0	0.5 to 1.5 cm.
	4	14	1.5	0.5 to 1.1 cm. roughened ducts.
	4	21	1.5	0.5 to 1.0 cm. roughened ducts.
Injected, spayed females	4	11	1/6	1 with end-buds, 2 interlobular ducts.
	4	20	1/6	2 with clumps of alveoli.
				2 with roughened ducts.
	4	14	0.5	2 with end-buds. No lobules. 2 roughened ducts.
	4	21	0.5	Ducts thickened with secretion. Interlobular ducts—No lobules.
	4	14	1.5	Roughened ducts. Glands rather small in 2 cases. End-buds 1 case.
	4	21	1.5	Thick roughened ducts—2 cases. End-buds—1 case. No lobules.

merely to that obtainable with the natural estrogens in the mouse. No true lobule development occurred, such as is found in pregnancy, even in spayed virgin females.

A total dosage of 0.05 γ in six days was reported to cause a mammogenic duct growth mouse unit response (12). In this study 0.167 to 0.5 γ per

day caused extensive duct development. Oral administration appeared to require about six times as much hormone as by injection.

Rats. Subcutaneous injection of stilbestrol into groups of three castrate virgin female rats at 0.004, 0.008, 0.017 and 0.034 gamma per day did not result in any obvious signs of mammary duct growth. Single glands were removed on the 7th, 14th, and 23rd days of treatment. The remaining glands were recovered and examined in toto after sacrificing on the 28th day (table 2). The mammary glands appeared to be in active rather than in regressed condition in most cases, however, staining deeply. Several glands removed had main ducts which were considerably thickened by side development of interlobular ducts and perhaps alveolar buds. No duct end-buds were apparent.

Three groups of similar rats given 0.25, 0.5 and 1.0 gamma per day of stilbestrol for 18 days all showed active proliferation of mammary ducts.

TABLE 2
Effect of stilbestrol on the mammary gland of the rat

Number of rats	Av. weight after treatment	Dosage, gamma per day	Results			
			7 days	14	23	28
	<i>gm.</i>					
3	177	0.004	Glands were all negative for additional duct growth.			
3	169	0.008				
3	191	0.017				
3	169	0.034				
			18 days			
3	111	0.25	Many duct end-buds and considerable proliferation of ducts.			
3	111	0.50				
2	106	1.00				

In this study four times the mouse unit dosage of stilbestrol gave no demonstrable signs of duct proliferation. Duct growth was obtained with a dosage of 0.25 γ per day which is at least 30 male mouse units (12). However, the minimum dosage to secure duct growth in the rat was not ascertained. This is in contrast to results secured with the natural estrogens in which the rat mammary gland appeared to respond to lower dosages than did that of the mouse (24, 25).

Rabbits. Twelve rabbits either injected or implanted with stilbestrol showed extensive mammary proliferation. Glands removed from males after injection of 4 to 32 gamma per day for 20 days had a complex duct system with an average extent of about 3 cm. (tables 3 and 4). This was not extensive development but glands from rabbits given 4 γ were as large as those from rabbits given 32 γ a day, indicating that 4 γ were adequate to secure the maximum rate of development. Since it appeared that the dosage might still be above the optimum, the dosage to four of the rabbits was then reduced to 1/10 that of the first 20-day period (0.4 γ –3.2 γ) and

single glands were removed by biopsy at 40 and 60 days. The 40-day glands averaged 4.4 cm. in extent and the 60-day glands 5 cm. The rabbits were sacrificed at 70 to 80 days of injection when all remaining glands averaged over 6 cm. They compared favorably in size with those of virgin female rabbits but were in several cases more complex. These glands consisted not only of duct systems but in addition had considerable lobule development (fig. 2).

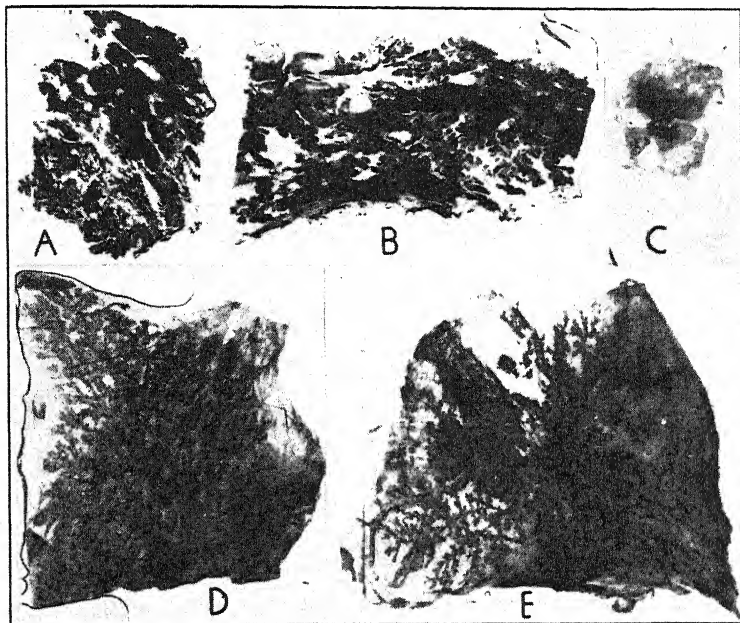


FIG. 2. a. Section of mammary gland from a virgin female rabbit (No. 24) given 32 γ daily of stilbestrol for 20 days. This gland contained copious quantities of milk when removed. There was a well developed lobule system with hypertrophied alveoli. $\times 1$.

b. Section of a mammary gland from a male rabbit (No. 29) with an 18.7 mg. pellet of stilbestrol subcutaneously. This gland was obtained 97 days from implantation of the pellet. The extensive lobule-alveolar system was expanded with milk from administration of a rabbit unit of lactogen. Compare with control gland (c). $\times 1$.

c. A typical control gland from a male rabbit. $\times 1$.

d. Half of a mammary gland from a male rabbit (No. 22) given 16 γ of stilbestrol daily for 20 days and then 1.6 γ for 45 days. Interlobular ducts are well developed and a few areas appear to have small lobules with alveoli. $\times 1$.

e. Mammary gland from a male rabbit (No. 14) given 32 γ of stilbestrol daily for 20 days and then 3.2 γ for 50 days. Lobules have developed in the center of the gland. $\times 1$.

An attempt was made to secure milk secretion from the small duct systems of four of the male rabbits after 20 days treatment with stilbestrol.

TABLE 3
Response of rabbit mammary glands to stilbestrol and lactogen

Sex	Mode stilb. admin.	Dosage stilb. /day 20 days	Lactogen treatment		Dia. 27-day glands	Type of development	Dosage stilb. /day 20 days	Lactogen treatment		Dia. 54-day glands
			Dosage	Response				Dosage 6 days	Response	
Male	5	Injection	32 γ	10 i.u./100 gm. body weight	Serous	Interlobular ducts				
"	14	"	32 γ		Serous	Early lobules				
"	4	"	16 γ		Serous	Ducts				
"	3	"	8 γ		Milk	Lobules				
"	13	"	4 γ		Serous	Ducts	8 γ	10 i.u./100 gm. body weight	Copious milk	5

cm.

cm.

Lactogen was given at the rate of 10 international units per 100 gm. body weight in six daily injections. At operation for removal of sample glands, those of three rabbits showed serous secretion and expanded ducts but no milk. Milk could be squeezed from the teats of the fourth rabbit and the small duct system was filled with milk. After 20 more days treatment with stilbestrol this rabbit had a 5 cm. duct system which again filled with milk on lactogenic treatment.

Three 1200 to 2500 gram virgin female rabbits injected with 8, 16, and 32 γ daily of stilbestrol had 5 to 7 cm. glands removed after 20 days of injection. The gland from the rabbit on 32 γ was engorged with milk in a lobule-alveolar system. The glands from the other two rabbits had no milk but the main and interlobular ducts present were swollen with serous secretion.

Glands removed at 40 days of injection did not show such obvious secretion. In two cases interlobular ducts were present while in the third early lobules were present. The glands removed after 60-65 days had in two cases unhyertrophied lobules. In the third only interlobular ducts were present.

Two male rabbits implanted with 16.8 and 18.7 mg. pellets of stilbestrol showed no mammary development in glands removed at 20 days after implantation (table 4). The 40-day glands removed were 4 to 5 cm. in extent, however, and showed what was apparently the beginning of lobules, perhaps consisting of intralobular ducts. Short central ducts were highly cystic. At 60 days the lobules were more apparent. Glands removed at 80 days appeared to have true lobules with alveoli. These were rather scattered and small and did not constitute the pseudo-pregnant gland condition. These glands were approximately 6 cm. in extent. At 90 days lactogen was administered at the rate of 12 i.u. per 100 grams body weight. The mammary glands became swollen with milk so that it could be expressed from the teats. Glands removed on the 97th day were composed of hypertrophied lobules of alveoli and ducts full of milk (fig. 2). Three glands removed from one rabbit at necropsy at 103 days averaged 9.5 cm. in extent. The second rabbit was sacrificed at 122 days when two 10 cm. lobule-alveolar glands were removed. These glands contained isolated areas composed of apparently abnormal lobules with very large alveoli, as seen by Gomez and Turner (23) after anol treatment of rabbits.

Five rabbits given 0.02 γ to 2.0 γ per day per teat applied in alcohol to the shaved skin for 30 days responded with growth of the mammary glands. Two of these rabbits which received 2 γ and 0.2 γ per teat had glands averaging 4.2 cm. and 4.7 cm. in extent. Some of these glands were over 5 cm. in diameter. Development had progressed to the interlobular duct or early lobule stages. The teats were also considerably enlarged over the control condition. This also occurred in the rabbits injected with stilbestrol and in those with pellets.

TABLE 4
Response of rabbit mammary glands to stilbo-sterol and lactogen

Sex		Mode stilb. admin.	Dosage stilb. /day	Dia. 20-day glands	Type of develop- ment	Dosage stilb.	Dia. 40-43 day glands	Type of develop- ment	Dia. 60-65 day glands	Type of develop- ment	Dia. 70-80 day glands	Type of develop- ment
Male	14*	Injection	32 γ	2.3	Lobules	gamma	cm.		cm.		cm.	
"	22	"	16 γ	3.2	Ducts	3.2	4.0	Lobules	2.4	Lobules	5.6, 5.7	Lobules
"	23	"	8 γ	2.5	Ducts	1.6	5.7	Interlobular ducts	5.5	Interlobular ducts	5.7, 6.1	Interlobular ducts
"	13*	"	4 γ	3.5	Ducts	0.8	4.0	Ducts	4.4	Plain ducts	6.6, 7.7	Early lobules
Virgin female	24	"	32 γ	6.6	Lobules Alveoli small Milk in ducts	0.4	4.0	Early lobules of alveoli	5.6	Ducts	6.4, 5.0	Interlobular ducts
"	25	"	16 γ	5.0	Ducts Serous secre- tion		5.7	Lobules of alveoli	8.2	Lobules		
"	26	"	8 γ	5.0	Interlobular ducts Serous secre- tion		6.0	Lobules	10.0	Lobules		
Male	28	Subcutaneous pellets	16.8 mg. Neg.				5.5	Swollen ducts	8.5	Ducts		
"	29	Subcutaneous pellets	18.7 " Neg.				5.0	Early lobules	4.6	Lobules	6.0	Lobules
							3.9	Early lobules	4.3	Early lobules	5.5	Early lobules
Lactogen treatment at 90 days												
			Dosage	Response	Dia. 97 day glands	Type of development	Dia. 103 day glands	Type of development	Dia. 122 day glands	Type of development		
Male	28 (continued)	12 i.u./100 gm. body weight		Copious milk	cm.		cm.					
"	29 (continued)			Copious milk	6	Cystic ducts Large lobules	8.8, 10.2 9.5	Lobules Small alveoli				
					12	Isolated lobules, hypertrophied					9.8 5.8, 10.0	Small alveoli Lobules

* Days elapsed between the two series of injections, rabbit #14-26 days; rabbit #13-40 days.

Male rabbits responded with extensive mammary development on injection of as little as 0.4 γ per day of stilbestrol or on percutaneous application to the shaved skin. It is interesting to note that in the male rabbit the lobule-alveolar system developed to a greater extent than in the female mouse similarly treated. Alveolar lobules began to appear after 20 to 40 days treatment in the rabbit and were well developed in rabbits implanted with pellets of stilbestrol.

Well developed mammary duct systems grown with stilbestrol in male rabbits came into lactation upon administration of lactogen. It was not necessary to terminate the stilbestrol treatment during lactogen administration for male rabbits with subcutaneously implanted pellets responded readily. Stilbestrol was thus shown to have the estrogenic property of preparing the mammary gland to respond to lactogen by the production of milk (26, for review).

A normal adult female rabbit did not require the administration of lactogen, for it responded with copious milk secretion to stilbestrol injection alone. This has also been shown to occur in the dry female goat (18, 19). Stilbestrol appears to cause the release of lactogen from the animal's own anterior pituitary in these cases, for a goat brought into milk with stilbestrol had at least twice the lactogen content in her urine as did normal dry goats (19).

Goats. Six goats were given subcutaneous injections of stilbestrol for periods from 96 days to 8 months. The substantial lactations which re-

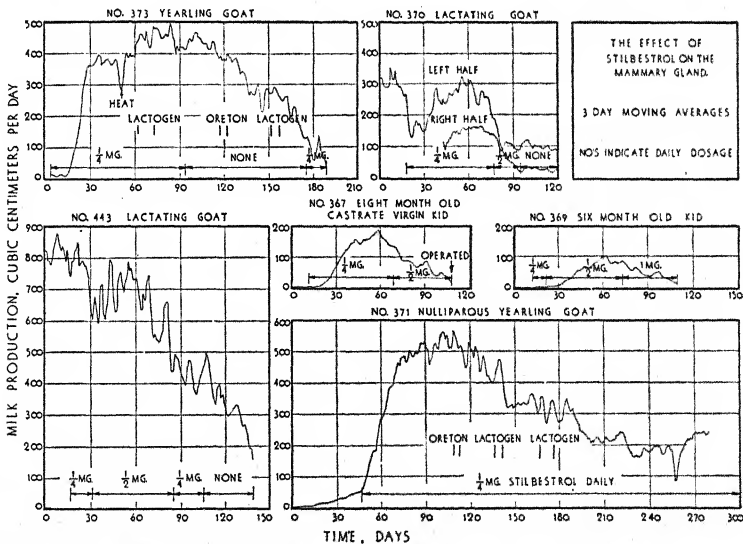


FIG. 3. The effect of daily, subcutaneous stilbestrol injection upon lactation in a castrate and a normal virgin kid, two yearlings goats which had never lactated and two mature goats in milk.

sulted from two kids and two yearlings has been previously reported as was the effect on the milk production of two goats which were already in lactation before the beginning of treatment (19). These lactations have been charted and are reproduced here (fig. 3).

The two virgin female kids received daily injections of stilbestrol for 96 days. The kids were six (No. 369) and eight (No. 367) months old when treatment was begun in July, so presumably they had had no estrous cycles. Goat No. 367 was castrated shortly before stilbestrol treatment was begun. Initial dosage was 0.25 mg. per day raised to 0.5 mg. on the 58th day; a total of 34.25 mg. in 96 days. Goat No. 369 was given 0.25 mg. per day for 12 days, 0.5 mg. for 44, 1.0 mg. a day for 41 days; a total of 67 mg. in 96 days.

At this time half of the udder was removed from each kid. The mammary glands were found to be about 4 cm. in diameter and consisted of ducts with thick clusters of lobules as in normal lactating glands after parturition, although most of the alveoli were no longer secreting heavily. Occasional alveoli or even lobules appeared to be still quite active (fig. 4). The lobules from the normal kid (No. 369) appeared to be more compact and fully developed than those from the castrate (No. 367). The extent of the glands does not appear to have been much increased but the lactation induced had caused the glands to expand into spherical form and had developed the gland cisterns. A mammary gland removed from a 37 day pregnant goat did not have nearly as complete a development of the lobules. Another gland removed from a 74 day pregnant kid appeared to have a complete lobule-alveolar system which was unhyertrophied, however (fig. 4). The alveoli were small masses of cells without lumina. This gland measured 0.5 cm. in thickness and 5.5 by 3.5 cm. in extent.

In contrast to the glands from stilbestrol treated kids Turner and Gomez (27) have shown that the mammary gland of the immature female goat consists of a thin, one centimeter or less, layer of ducts lying at the base of the teat and extending a few centimeters from it. Gland sections showed that the mammary system although rather complex consisted of a two cell layered duct system without lobules. Short, wide branches of the smaller ducts which gave the impression of alveolar buds were also two layered except the distal ends which were composed of solid masses of epithelial mammary cells.

A mammary gland removed from a mature male castrate goat (No. 833) showed no development after extensive stilbestrol treatment (fig. 4). For 78 days 0.25 mg. of stilbestrol was given daily by injection, a total of 19.5 mg. The teats were then enlarged considerably. Twice daily milking was instituted on the 52nd day but the yield was only 0.5 to 1 cc. per day for 10 days. No treatment was given from the 79th to the 118th day. Then three small hard pellets of stilbestrol, a total of 71.75 mg., were implanted sub-

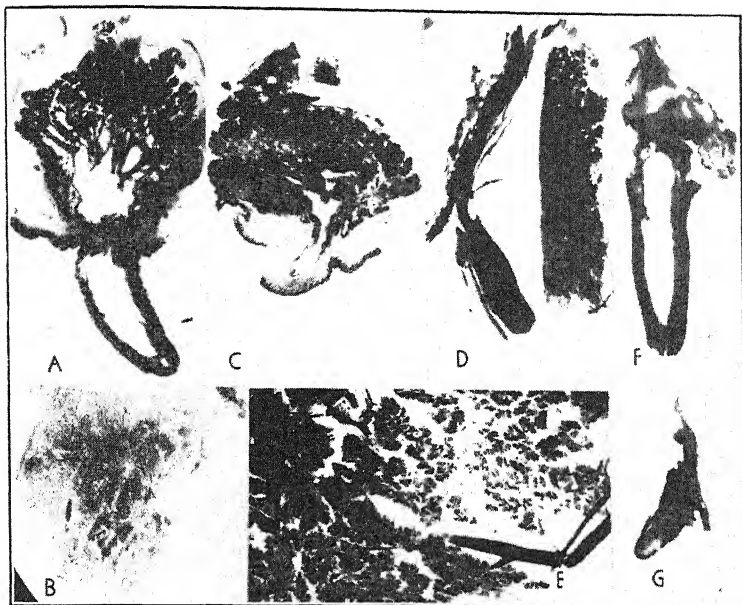


FIG. 4. a. Mammary gland section from castrate virgin kid 367 after 96 days treatment with stilbestrol. There is a well developed lobule alveolar system. The teat is enlarged. $\times .64$.

b. Enlarged microsection from mammary gland of goat 367 showing hypertrophied alveoli. $\times 3$.

c. Mammary gland section from virgin kid 369 after 96 days treatment with stilbestrol. This gland is similar to that from goat 367 except that it appears more compact partially because no fluid was injected into the gland during fixation. The teat section here is incomplete. The teat from this gland was equal in size to that of goat 367. $\times .64$.

d. Dorso-ventral section with teat from gland of a 74 day pregnant kid and a lateral section from the same gland. This gland was flat compared with the globular shape of glands from goats 367 and 369. $\times .64$.

e. Enlarged section from gland of 74 day pregnant kid showing lobules. The alveoli in this gland were unhyertrophied clusters of cells. $\times 3$.

f. Mammary gland and teat from a mature castrate male goat after 78 days injection of stilbestrol and 75 days with 71.75 mg. of subcutaneous pellets. The teat is obviously enlarged but the gland consisted of a short duct and a cluster of mammary cells at the base of the teat. $\times .64$.

g. Section of teat and mammary gland from an untreated castrate male goat. Compare size of teat with that in (f). The mammary gland consists of two very small clusters of cells at the base of the teat. $\times .64$.

cutaneously. A mammary gland was removed on the 292nd day which consisted of a teat cistern and a small clump of mammary cells a few millimeters in diameter. The absorption rate of 100 mg. stilbestrol pellets in women was found to be 0.127 to 0.25 mg. per day and they were effective for 400 to 800 days. Pellets were reported to be 5 to 10 times as effective

as injection on a dosage basis (5). Proportionally 0.09 to 0.18 mg. of stilbestrol should have been available to cause mammary development in this male goat but none occurred. The teat had developed, however, for it measured 4.5 cm. in length and was more than twice the length of that found in control male goats.

Turner and Gomez (27) found that the mammary gland of a six-months-old male goat was only one centimeter in diameter. That from a four-year-old male was 5 cm. in extent. The duct system extended little beyond the base of the teat, however. In exceptional males the glands may attain considerable development and may even lactate. A gland removed in this study from a ten-months-old male goat castrated at 8 months of age consisted of a teat cistern and a very small cistern at the base of the teat (fig. 4). This cistern was surrounded by several layers of epithelial mammary cells the extent of which was under 0.5 cm. The teat was two centimeters in length.

This study has shown that stilbestrol caused little duct development but extensive lobule-alveolar hyperplasia in a castrate and a normal kid treated subcutaneously for 96 days. Abundant and prolonged lactation occurred. Extensive stilbestrol treatment both by injection and with pellets in a mature castrate male goat failed to cause mammary development. Only the teats developed as did those of the kids treated.

DISCUSSION

Stilbestrol proved to be a very active mammary duct growth factor in mice, rats and rabbits. It was rather surprising to find that in female goats treated for 96 days instead of extensive duct growth there had occurred the proliferation of rather restricted lobule-alveolar systems. Early lobule development was found in the injected male rabbits and more extensive development in those with pellets but this only occurred after extensive duct growth. The ovaries could hardly have been a primary factor in the lobule development in goats, for one of the kids had been castrated before initiation of treatment. For the same reason the condition of the ovaries was probably not instrumental in initiating, through action on the pituitary, the lactation which occurred in these and the yearling goats. In fact the castrate kid responded more readily than the normal one and produced a greater amount of milk.

It remains to be seen whether extensive duct growth can be obtained in the goat with stilbestrol. In laboratory animals the male responds readily to estrogen treatment with proliferation of mammary ducts. The failure with stilbestrol in the male goat treated may have been due to inadequate dosage.

SUMMARY

Subcutaneous injections of stilbestrol at low dosages caused extensive

duct proliferation in male mice in 2 to 4 weeks. Mammary development did not proceed farther in spayed virgin female mice similarly treated. Oral administration of stilbestrol to male mice required approximately six times as high a dosage as by injection to obtain similar results.

Castrate male rats required a higher dosage of stilbestrol than did mice to obtain mammary duct growth.

Four-tenths gamma per day of stilbestrol subcutaneously was adequate to secure extensive mammary duct development in male rabbits. After 40 to 60 days treatment early lobule development was apparent. Percutaneous administration was also effective. Mammary glands from two rabbits with subcutaneous pellets had well developed lobule-alveolar systems and responded well to lactogen treatment at 90 days. Normal females tended to lactate on stilbestrol injection alone.

Subcutaneous injection of stilbestrol into virgin goats caused abundant and prolonged lactation from lobule-alveolar glands. Little increase in extent of glands was apparent. Subcutaneous administration followed by pellet implantation caused no mammary gland development in a castrate male goat, although the teats were hypertrophied.

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THE CONTENT OF GRASS-JUICE FACTOR IN LEGUME SILAGES AND IN MILK PRODUCED THEREFROM*

B. CONNOR JOHNSON, C. A. ELVEHJEM AND W. H. PETERSON

Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison

In previous publications (1, 2, 5) from this laboratory it has been shown that many plant materials contain an unidentified water-soluble growth-promoting substance for rats and guinea pigs. Because of its abundance in young grass this substance has been called "grass-juice factor." Johnson *et al.* (3) found that this factor can be preserved by suitable methods of ensiling and that cows fed such silage produced a milk rich in the factor. In this paper are reported additional data on the effectiveness of various methods of ensiling for preservation of the factor and also data on the growth-promoting quality of the milk from cows fed a number of these silages.

EXPERIMENTAL

Guinea pigs weighing approximately 300 gm. each were fed a basal diet of mineralized milk supplemented with riboflavin as reported in the previous publication (3). This milk was obtained from cows that had been fed for several months a winter ration which was low in the grass-juice factor.

Silages. The silages fed were preserved in the various ways listed in table 1. Three types of containers were used: quart milk bottles, 40 and 50 gallon barrels, and regular silos.

Samples of the fresh forages at the time of cutting were dried 24 hours at 40° C., ground and stored in the refrigerator. When the bottles, barrels, and silos were opened, samples of the silages were dried, ground and stored in the same way.

Guinea pigs, usually two or more animals per group, were fed 3 gm. per day of the dried materials as a supplement to the basal diet. Orange juice was added to the dried silages to increase the palatability. Weight gains of animals receiving these supplements are given in table 1.

Milks. The growth-promoting qualities of milk produced by cows fed some of the preceding silages were determined. Three groups of five cows each were included in the experiment. Groups I and II each contained two Holsteins, two Guernseys and one Brown Swiss; Group III consisted of two Holsteins and three Guernseys. The ration was 39 lbs. of silage, 8 lbs. of alfalfa hay, and 8 lbs. of grain mixture per cow per day. Oats-peas silage made in different ways was fed to group I for varying periods of time as

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follows: molasses-preserved silage, 4 months; untreated silage, 5 weeks; phosphoric-acid-treated silage, 7 weeks. Alfalfa silage prepared with 20 lbs. of phosphoric acid per ton was fed to Group II for 4 weeks, the same forage prepared with 14 lbs. of acid per ton was fed for 5 weeks, and that with 8 lbs. of acid per ton was fed for 10 weeks. Group III received molasses-alfalfa silage for 5 months.

Each day all the milk from one milking of the cows in a group was mixed together, and a quart sample was taken. This sample was fed to a group of 3 or 4 guinea pigs. A small amount of the milk plus 1 mg. iron, 0.1 mg. copper and 0.1 mg. manganese was fed first in the morning, and after this had been consumed, an excess of milk was given. The average growth curves are plotted in figure 1. The growth curve of a control animal fed the basal winter milk is also included in the figure.

DISCUSSION

The increases in weight of the guinea pigs (table 1) show that the grass-

TABLE 1

Assay of silages for grass juice factor

Forage ensiled	Preservative used in ensiling		Weight gain of guinea pigs in 7 weeks
	Kind	Amount per ton	
None	<i>gms.</i> - 30
Alfalfa	Fresh (not ensiled)	123
Alfalfa	None	22
Alfalfa	Molasses	60 lbs.	47
Alfalfa	Salt	10 lbs.	44
Alfalfa	Phosphoric acid	15 lbs.	88
Alfalfa	Soured whey concentrate* equal to whey at	600 lbs.	141
Alfalfa	Whey powder	80 lbs.	61
Clover-timothy (1-1)	Fresh (not ensiled)	98
Clover-timothy (1-1)	None	16
Clover-timothy (1-1)	Molasses	60 lbs.	50
Clover-timothy (1-1)	Phosphoric acid	30 lbs.	93
Oats-peas (1-1)	Fresh (not ensiled)	76
Oats-peas (1-1)	None	36
Oats-peas (1-1)	A.I.V. acid mixture	34 litres 2N acid	74
Oats-peas (1-1)	Molasses	60 lbs.	89
Oats-peas (1-1)	Phosphoric acid	20 lbs.	108
Soybean	Fresh (not ensiled)	155
Soybean	None	114
Soybean	Molasses	100 lbs.	103
Sudan grass	Fresh (not ensiled)	93
Sudan grass	Molasses	40 lbs.	70

* Soured by *L. bulgaricus*.

juice factor of the forage was retained in varying degrees by different methods of ensiling. Acid-prepared silages were somewhat superior to molasses-

preserved silages in growth-promoting quality. With the exception of soybean silage, silages prepared without added preservative were rather low in the grass juice factor. Alfalfa preserved with soured whey concentrate gave especially good growth. Of the forages tried, soybean was the richest in the grass-juice factor.

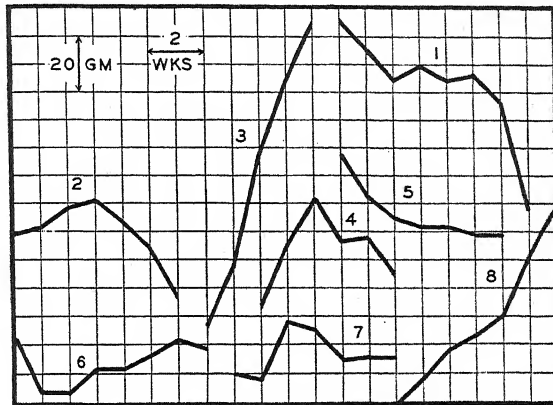


FIG. 1. Average growth curves of guinea pigs receiving various mineralized milks as follows: No. 1, control basal winter milk; No. 2, molasses alfalfa silage milk; No. 3, phosphoric acid alfalfa silage milk (20 lbs. acid per ton); No. 4, as No. 3 at 14 lbs. per ton; No. 5, as No. 3 at 8 lbs. per ton; No. 6, molasses oats-peas silage milk; No. 7, no preservative oats-peas silage milk; No. 8, phosphoric acid oats-peas silage milk (12 lbs. acid per ton).

From figure 1 it can be seen that good growth was obtained with milk produced from alfalfa silage that had been prepared with 20 lbs. of phosphoric acid per ton, but the use of smaller amounts of phosphoric acid resulted in poor quality milks. As reported elsewhere (4) better preservation of other constituents in the silage was obtained with the higher amount of phosphoric acid. With oats-peas 12 lbs. of acid per ton was sufficient to insure the presence of the factor in the milk but when this forage was ensiled with molasses, the milk produced from it was of low potency.

SUMMARY

The grass-juice factor of forages can be preserved in silage but the extent of preservation varies with different methods of ensiling. Silages prepared with phosphoric acid contained somewhat more of the factor than molasses-treated and untreated silages. Addition of soured-whey-concentrate to alfalfa gave excellent preservation.

The quantity of grass-juice factor in winter milk was increased by feeding silages rich in the factor, *e.g.*, silages preserved with adequate amounts of phosphoric acid.

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THE RELATIONSHIP OF pH TO SOME CURD CHARACTERISTICS OF MODIFIED MILKS

ARNOLD B. STORRS

American Seal-Kap Corporation, Long Island City, N. Y.

In recent studies of the digestibility of milk several *in vitro* tests have been proposed in which stress has been laid upon the property of the curd particle size or the curd surface area. In the Chambers-Wolman test (1, 2, 3) the curd surface area is the index by which digestibility is gauged. In other methods reported by Hull (4) and Flora and Doan (5) although the measurement of protein degradation has been the basis of determining digestibility the importance of the size of the curd particles has been evident.

Among the factors which may influence the type of curd formation the pH at which coagulation occurs and the range of pH throughout digestion are of significance. This has been noted by several investigators (3, 4, 6). In the *in vitro* tests suggested, however, there have been considerable differences in technique with respect to the pH levels employed and possibly this has been a matter of greater controversy than any other single factor.

Available published reports concerning the conditions of acidity in the human stomach offer little to clarify the situation. The methods employed and the results obtained by several workers (6 to 15 inclusive) have differed so widely that the data appear too variable to warrant the formation of any definite conclusions.

Thus if the effect of pH on the coagula of different types of milk was proportionately the same it would make little difference what pH level was selected for *in vitro* tests. Preliminary work by the author indicated that some modified milks might react differently than others to pH changes. This investigation was undertaken to ascertain the behavior of various commercially modified milks at different pH levels.

EXPERIMENTAL

Samples: All samples used in the study were commercially prepared and included the following types of milk:

Untreated milk, both raw and pasteurized.

Homogenized milk (high pressure, piston type homogenizer).

Enzyme-treated milk (pancreatic enzyme extract).

Base exchange milk.

Evaporated milk, diluted 1:1 with water.

Curd surface area measurements: The Chambers-Wolman test (1, 2) with the modified technique as described by Anderson (3) was used for measurements of curd surface area. The samples were coagulated in thin-

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walled latex sacs, using sufficient coagulant to adjust each to the desired pH level. The coagulant consisted of a mixture of equal parts of 0.6 per cent pepsin solution (U.S.P. 1:3000) and normal hydrochloric acid. After a digestion period of thirty minutes with constant agitation the samples were emptied into individual containers and hardened with formaldehyde. After sieving and weighing the various size fractions of curd particles the relative surface area per gram of curd (S/gm.) was determined by calculation. The results reported are the averages of duplicate tests.

Measurements of total curd formation: In the Chambers-Wolman test the measurement of the total amount of curd formed is a necessary step in the calculation of the curd surface area. The same data were used in this investigation as a method of studying the relative bulkiness and completeness of curd formation at different pH levels. As a further guide to the completeness of coagulation the appearance of the samples at the end of the "digestion" period was observed. Complete coagulation was considered to have occurred only when there was a definite and complete separation of the sample into coagulum and whey.

pH values: A Beckman pH meter with remote electrodes was used and all readings were made at a temperature of 25° C. Each sample was tested at the following pH levels: 6.0, 5.5, 5.0, 4.5 and 4.0.

Total solids: Total solids were determined by means of a lactometer and butterfat tests.

All tests on any given sample of milk were performed on the same day.

RESULTS

Figure 1 shows the average amounts of curd recovered from the different types of milk in the Chambers-Wolman test throughout a pH range from 6.0 to 4.0.

The untreated, homogenized and enzyme-treated milks yielded somewhat similar results with the smallest amount of curd being recovered in each case at pH 5.0. Of these three types of milk the homogenized varied the least and tended to form a somewhat bulkier curd throughout the entire range. The amount of curd formed by the untreated milk was greatest at pH 6.0, dropped to its lowest point at pH 5.0 and then increased slightly as the pH was lowered further. The enzyme-treated milk showed the greatest variation and formed considerably smaller amounts of curd than the other two at pH 5.5 and pH 5.0. As judged by the amounts of curd recovered and the appearance of the samples these milks all coagulated completely throughout the pH range studied.

In base exchange and evaporated milk the effect of incomplete or partial coagulation was noticeably demonstrated. It is an established fact that these milks do not form any appreciable curd under the conditions of the curd tension test which is carried out at about pH 6.0. Likewise, when the

Chambers-Wolman test was performed at pH 6.0 the base exchange milk averaged 7.14 grams of curd while the evaporated milk did not form any curd. As the pH was lowered both types coagulated completely. In the case of base exchange milk this point was reached at about pH 5.5 and with evaporated milk at about pH 5.0.

Table 1 shows a comparison between the total solids content and the average amounts of curd recovered. This was done as a check against the effect of possible variations of the total solids of the different milks upon the amounts of curd formed in the Chambers-Wolman test. It is evident from the data that there was little relationship if any between the solids content of the milks and the amounts of curd recovered.

TABLE 1

The average total solids and the amounts of curd recovered in the Chambers-Wolman test at different pH levels

Type of milk	No. of samples	Total solids	Amount of curd recovered				
			pH 6.0	pH 5.5	pH 5.0	pH 4.5	pH 4.0
		%	gms.	gms.	gms.	gms.	gms.
Untreated	11	13.47	40.98	34.35	27.79	31.50	35.33
Homogenized	10	12.98	42.37	36.52	36.07	37.95	40.17
Enzyme-treated	11	13.03	39.21	26.25	23.17	33.62	38.50
Base exchange	10	12.57	7.14	30.13	30.47	32.33	39.08
Evaporated (1:1)	10	13.78	0.00	27.64	52.54	54.17	52.09

In figure 2 are shown the average values for curd surface area throughout a pH range from 6.0 to 4.0. Also in this respect the untreated, homogenized and enzyme-treated milks followed the same general pattern with the values being low at pH 6.0 and increasing as the pH decreased. At pH 6.0 and 5.5 all three types of milk gave approximately the same low values. It was only at pH 5.0 or lower that there were appreciable differences.

The results with base exchange milk differed from the three just mentioned in that the curd surface area was comparatively high at pH 6.0 and dropped to its lowest point at pH 5.5. Undoubtedly this high surface area was due to partial coagulation of the milk. At pH 5.0 the curd surface area was approximately equal to that of the untreated milk and, while it increased somewhat as the pH decreased, base exchange milk still had the lowest surface area of all samples at pH levels of 4.5 and 4.0.

With respect to curd surface area the evaporated milk was superior to all types tested. No curd was formed at all at pH 6.0 while at pH 5.0 the values were generally very high. As with base exchange milk the high surface area at the upper pH levels coincided with partial coagulation. The lowest curd surface area occurred at pH 5.0 from where it increased slightly at pH 4.5 and showed a much greater increase at pH 4.0. Five different brands of canned evaporated milk were included in the study. There ap-

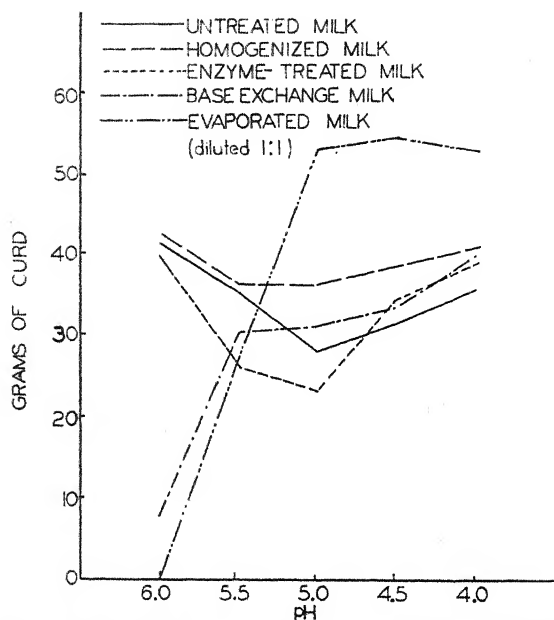


FIG. 1. The total amounts of curd recovered in the Chambers-Wolman tests throughout a pH range from 6.0 to 4.0. (Averages of all samples of each type of milk.)

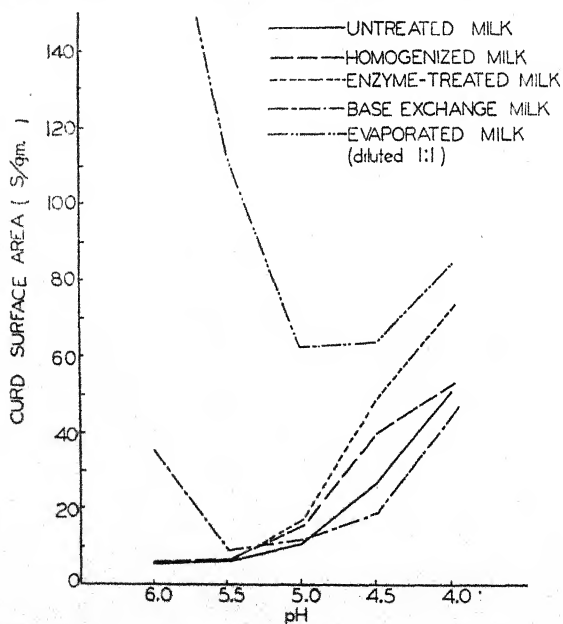


FIG. 2. The average curd surface area of modified milks throughout a pH range from 6.0 to 4.0.

peared to be some similarity of results within brands. However, the numbers of samples of each brand tested were too small to permit the formation of definite conclusions.

DISCUSSION

Considering the data with respect to the total amounts of curd formed in the Chambers-Wolman test it becomes apparent that there are two principal factors which will affect the results: 1) the completeness of coagulation and 2) the hydration of the curds or their affinity for water. Since there were no adequate means available for measuring either of these in a quantitative manner no attempt to do so was made in this investigation.

The completeness of coagulation was judged almost entirely by the appearance of the samples after "digestion" and was interpreted as being "no coagulation," "partial coagulation" or "complete coagulation" depending upon whether there was no curd formation, some curd formation or a complete separation of curds and serum. Under the conditions of the tests incomplete coagulation was observed only with base exchange and evaporated milks within the pH range studied. While the untreated, homogenized and enzyme-treated milks all coagulated completely at pH 6.0 and lower, it seems logical to expect that if even higher pH levels had been employed a zone would have been found in which they too would have exhibited varying degrees of partial coagulation. This statement is based upon the assumption that if coagulant were added to the milk in increasing increments it would be expected that coagulation would occur gradually.

The values reported as the total amounts of curd recovered in the Chambers-Wolman test are "wet" weights and therefore any variations in the hydration of the curd formations would have a corresponding effect upon results. While no specific method of measuring the degree of hydration was employed there seems to be no other logical explanation that could be offered for the variations in the amounts of curd formed at different pH levels in those samples wherein coagulation was complete. Certainly the total solids content (Table 1) was of little importance and it is not likely that at the pH levels employed there was any actual peptic digestion of consequence. With respect to the effect of pH upon the total curd formation, the general reaction seems to be determined by the inherent physical and chemical properties peculiar to each type of milk as a result of the method of modification employed.

When considering tests run throughout a range of pH levels there seemed to be a general relationship between the amount of curd formed in the Chambers-Wolman test and the curd surface area. This was particularly true at the upper pH levels where coagulation of the samples was more likely to be incomplete. Attention has been called to the effect of partial coagulation upon the values for curd surface area in the case of the base exchange and evaporated milks. It is interesting to observe that after these milks had

coagulated completely their values for surface area increased with a decrease in pH in much the same manner as the untreated, homogenized and enzyme-treated milks. Therefore, in the relationship of pH to curd characteristics one fact seems to hold true for all milks, *i.e.*, the curd surface area is lowest at the highest pH level at which complete coagulation will first occur. In the case of the untreated, homogenized and enzyme-treated milks this point is apparently reached somewhere above pH 6.0 and with base exchange and evaporated milk it is at about pH 5.5 and 5.0 respectively. Also, at any pH below that required to bring about complete coagulation, the curd surface area increases as the pH is lowered.

It has already been mentioned that there has been considerable variation in the *in vitro* techniques used by several investigators. This probably has been due to a lack of definite and conclusive knowledge of the coagulating conditions within the stomachs of human beings. Inasmuch as wide variations in the coagulating characteristics of milks occur within the pH limits thus far reported there does not seem to be any single pH level that is satisfactory for comparative tests on all milks.

CONCLUSIONS

In the Chambers-Wolman test the curd surface area of any milk appears to be lowest at the highest pH level at which complete coagulation will first occur. At any pH below that required for complete coagulation the curd surface area increases as the pH is lowered.

The effect of pH upon the bulkiness or completeness of curd formation is variable in milks modified by different processes. The method of modification seems to be the most important factor in determining this relationship.

With our present knowledge there does not seem to be any single pH level suitable for comparative *in vitro* tests on all milks.

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OBSERVATIONS ON DELAYED SALTING OF BRICK CHEESE¹

W. L. LANGHUS AND W. V. PRICE

University of Wisconsin, Madison, Wisconsin

A few Brick cheese manufacturers have attempted to hasten the ripening of Brick cheese by delaying for several days the normal salting operation which usually occurs in the morning of the day following manufacture. Brick cheese must be salted after it is shaped into its characteristic form. Salting is accomplished by exposing the surfaces of the loaves to sodium chloride brine or to dry salt.

Examination of the literature on the salting of cheese shows that the flavor, body, texture and color of cheese, its rate of ripening and its composition can be affected by the method of salting, the amount of salt used, and by the time of salting.

The flavor of cheese can be affected unfavorably by unusual salting methods. If the cheese lacks salt, undesirable flavors may be produced (1, 4, 14). Bitterness in cheese has been attributed to a protein decomposition product, the presence of which can be traced directly to the salt content (20). It has been suggested (13) that the inhibiting effect of salt on the growth of lactic streptococci might explain the relation between salt concentration in cheese and cheese quality. The flavor developed during curing can be decreased by over-salting (1, 9, 17).

The body of cheese is sensitive to variations in salt content. Generally, over-salting tends to produce a hard, harsh body (1, 7, 17) while under-salting gives a pasty, weak body (1, 17). There is a range of concentration through which the salt content can be varied without noticeably affecting the body of the cheese (9); this range probably will vary with other factors affecting body such as type of cheese, moisture content and acidity. Studies of the peptizing effect of salt on rennet casein under different conditions of salt concentration and acidity indicate that the smoothness of Cheddar cheese should be favorably affected by the action of the salt on the paracasein in the pH zone between 5.5 and 6.0 (18). On the other hand, the fact that cheese protein is 100 per cent soluble in 3 to 10 per cent sodium chloride solutions within 7 days after making and remains soluble throughout the life of the cheese seems to justify the conclusion that the influence of salt on cheese quality cannot be caused by variation in the solubility of the protein in brine (12).

The texture of cheese can be made open by light salting and close by heavy salting (1, 4, 17). A white discoloration may be induced in some soft,

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ripened types of cheese by over-salting (14), and a similar fault has also been observed in Brick cheese (1).

Salt affects organisms inside and on the outside of cheese. Bacterial growth inside the cheese is delayed by early salting (3) and by variations in the amount of salt incorporated (4, 14). The presence of salt on the outside of types of cheese not unlike Brick seems to encourage the development of surface flora (7, 8).

The method of salting may influence the results obtained. Dry salting apparently reduces the weight of the cheese more than brine salting (2) and is not apt to be as uniform as brine salting (1). Salting cheese on the outside by either of these methods after the cheese is formed produces results not observed when the curd is salted before it is shaped (9).

The effects of salting on cheese characteristics may be associated with the rate of salt penetration. It has been observed that in Edam cheese 15 cm. in diameter the salt penetrated 5.5 cm. in 10 days (22); in Limburger, 8 to 10 days are required for practically uniform distribution (5, 15). Brine salted, whole milk Trappist cheese weighing 1.2 to 1.3 kg. showed uniform salt distribution in 60 days (16); and salt applied to the surface of Brick cheese requires approximately 8 weeks before it is uniformly distributed (1). Salt diffuses slowly in Cheddar cheese and penetrates most easily along the "grain"; within 12 hours after hooping the salt is essentially uniform (11), although the distribution of salt in cheese from the same vat may be surprisingly lacking in uniformity (10).

Some observations have been made on the influence of the time of salting certain types of cheese. In one study (3), one portion of curd was salted as soon as it was drained while identical curd was pressed 2 days and then salted in a brine bath. The early salting retarded bacterial development; delayed fermentation of lactose and acid development; and when there was incorporated more than 4 per cent of salt in the moisture of the cheese the cheese became hard, brittle and crumbly. The cheese salted after 2 days could absorb 8 per cent salt in the water of the cheese without injury. Salting curd 3 hours after draining gave results similar to those obtained by salting after 2 days. Other workers (8) reported that salting of Camembert cheese 1 day after making increases the dry matter; delays acid formation; increases the salt content; and hastens development of surface flora as compared to salting 2 days after making. The late salting causes slow salt penetration and, because the curd retains the whey, there is induced bitter flavor and whey sourness. When Brick cheese (1) is salted at 4 hours after dipping instead of the usual 20 hours, almost all of the characteristics of the cheese are affected. The acidity development is practically stopped, more moisture is retained; salt penetration is more rapid; and, although the flavor is not materially altered, the body of the early salted cheese tends to be curdy and hard and the texture is closer.

It is clear that salt influences the characteristics of cheese. The growth of organisms is affected by the salting treatments; the chemical substances produced in the course of the ripening process are determined in part by the salt; and the physical properties of the protein are influenced by the combined action of acidity and salt content. The smoothness of body observed after delayed salting treatments and the harshness of body induced by early salting treatments are particularly significant in the making and curing of Brick cheese because this is a type of cheese which is most popular when the body has smoothness and good slicing properties. A few preliminary trials in this laboratory indicated that delayed salting induced differences which should be studied. The results of this study are reported here.

EXPERIMENTAL PROCEDURES

The manufacturing process used in these experiments was essentially that recommended by Spicer and Price (19). The experiments were made with raw and with pasteurized milk. Milk cultures of *S. lactis* were used for starters and a cooking temperature of 104° F. was therefore adopted.

The loaves of cheese from each lot of milk used in these experiments were divided into groups before salting. The cheese made from raw milk was divided into two groups one of which was salted in the usual manner on the day after making while the experimental group was salted on the 5th day after making. The cheese made from pasteurized milk was divided into three groups; the first or control group was salted the day after making; the second was salted on the 5th day; and the third group was salted on the 9th day after making. Each group was salted by holding in 23 per cent sodium chloride brine for 48 hours. All groups were kept at approximately 60° F. during salting, during the usual washing and until paraffining. All groups were paraffined on the 14th day after making and were then held at approximately 50° F. until the final grading.

Analyses for moisture (21) and acidity (pH) were made at 21 hours, just before salting, at paraffining and again when the cheese was 10 weeks old. Acidity measurements were made with a Leeds Northrup portable potentiometer using the quinhydrone electrode and saturated calomel half cell. Salt determinations (21) were made after salting and at paraffining and again when the cheese was 10 weeks old. These analyses were made by using the whole of a cross section slice of a loaf of cheese after discarding about $\frac{1}{4}$ inch of the rind layer. During the 5- and 9-day intervals before salting, the loaves of cheese were held in the 60° F. curing room and were moistened daily with water and rubbed to prevent mold growth.

All lots of cheese were graded at 14 days and again at 10 weeks of age.

RESULTS

The quality of the cheese is shown by the average grades listed in tables 1 and 2.

In table 1 are shown the grades of the 5 lots of cheese made from raw milk that were subjected to the different salting treatments. At two weeks of age the average quality of the cheese salted on the first day after making was practically identical except perhaps in flavor to that of the cheese salted five days after making. After 10 weeks of aging there was evident a slight margin of difference in favor of salting on the first day after making. The quality of the milk used in these experiments was not very good. All lots of cheese were criticized for off, sharp or unclean flavors after 2 weeks and for very unclean and strong flavors after 10 weeks of curing, regardless of the salting treatments.

TABLE 1

Effect of delayed salting on the quality of five lots of raw-milk cheese

Characteristic	Time of salting	
	1st day	5th day
Average grades* at 14 days of age		
Flavor	3.5	3.7
Body	2.4	2.5
Texture	3.7	3.6
Average grades* at 10 weeks of age		
Flavor	3.4	3.8
Body	3.2	3.4
Texture	3.2	3.4

* 1 = Excellent; 2 = Good; 3 = Satisfactory; 4 = Objectable; 5 = Very Objectable.

TABLE 2

Effect of delayed salting on the quality of three lots of pasteurized-milk cheese

Characteristic	Time of salting		
	1st day	5th day	9th day
Average grades* at 14 days of age			
Flavor	2.7	2.7	2.3
Body	2.0	2.3	1.3
Texture	2.5	2.7	1.8
Average grades* at 10 weeks of age			
Flavor	3.3	3.0	3.7
Body	2.7	2.3	2.3
Texture	2.7	2.3	2.3

* 1 = Excellent; 2 = Good; 3 = Satisfactory; 4 = Objectable; 5 = Very Objectable.

Early gas, evidently caused by organisms of the *Escherichia-Aerobacter* group, was present in every lot of raw-milk cheese regardless of the salting treatment. No definite relation could be observed between the time of salting and the degree of openness in the texture of the cheese. Despite the fact that lack of salt during the first 2 weeks of curing encourages the development of the splitting defect (4) in Brick cheese, this fault was not observed in the raw-milk cheese. The judges found a softness of body in the raw-milk cheese

salted 5 days after making which was not apparent in the cheese salted the day after making. This softness was of such a character that it was regarded as a defect.

The data of table 2 show the grades given the groups of cheese made from pasteurized milk. The grading of the cheese when it was 14 days old showed little difference in quality between those groups salted on the first and 5th day after making. The cheese salted on the 9th day was definitely better than that in the other groups. This improvement was found chiefly in a very desirable smooth, long body and a closer texture in the cheese. The delay in the salting operation for 9 days seemed to cause a more rapid disappearance of the normal curdy characteristics of the cheese. There was a slight improvement in flavor which was evident as a sweet or Swiss-like aroma in some of the lots salted 9 days after making.

After 10 weeks of curing the differences in quality between the groups observed when the cheese was younger had practically disappeared. The quality of all groups of cheese was regarded less favorably by the judges. These trends can probably be attributed to the quality of the original milk from which the cheese was made. The defects observed in the flavor were found in all three groups of cheese but the cheese salted 9 days after making had deteriorated most markedly. Regardless of the salting treatment all groups of cheese showed some mealiness of body. The texture of the cheese salted at 5 and 9 days after making was inferior to that of the control lot. Especially significant was the fact that the cheese salted on the 9th day showed some splitting.

The differences in quality recorded in tables 1 and 2 as the result of delayed salting might be caused by either biological or chemical changes or, more probably, both. There can be little doubt that delayed salting permitted the early and rapid growth of organisms which are ordinarily suppressed by the presence of salt. The marked differences in the body of the cheese, especially in the early stages of curing, reflect the effect of salt on the physical and chemical changes in the protein. It is well known in the industry that salt causes a firmness in cheese that cannot be attributed entirely to a lowered percentage of moisture. In addition to this effect, differences in the experimental cheese may be caused indirectly by the probable influence of salt upon normal acidity changes which must precede the breakdown of cheese curd.

The amount of salt in the cheese. The total amount of salt present in the cheese 10 weeks after curing was only slightly affected by the delay in salting as shown in table 3. The slightly higher concentration in the control lots can be explained by the use of 10 per cent sodium chloride brine to moisten the cheese during the interval of "smearing" before paraffining. Those lots given the delayed salting treatment received brine rubbing only after they had been salted; before salting only fresh water was used on them

for smearing purposes. It seems apparent that delaying the salting treatment influences the final salt content of the cheese so little that differences in the cheese must be primarily associated with the time interval between making and salting.

TABLE 3
Effect of delayed salting on the salt content of Brick cheese

Time of analysis	Time of salting		
	1st day %	5th day %	9th day %
Raw-milk cheese*			
After salting	1.43	1.06
At 14 days	1.90	1.76
At 10 weeks	1.90	1.82
Pasteurized-milk cheese**			
After salting	1.12	1.08	0.84
At 14 days	1.66	1.67	1.56
At 10 weeks	1.73	1.67	1.67

* Average values for 5 lots of raw-milk cheese.

** Average values for 3 lots of pasteurized-milk cheese.

Cheese acidity. The effect of delayed salting on the acidity of the cheese is shown in table 4. These results if considered alone would lend support to the belief that delayed salting would hasten the ripening process. The curing of cheese is accompanied by an increase in pH value; such results are slightly apparent in the trend of data shown for the pasteurized-milk cheese and a little more apparent in the data on acidity changes in the raw-milk cheese. The differences are so small however that they can be disregarded, especially in view of the meager supporting evidence obtained in examining the quality of the cheese.

The pH values shown for the raw-milk cheese are higher at the 10-weeks' interval than those shown for the pasteurized-milk cheese. This result is to be expected in view of the decreased biological activity in the curing process following the heat treatment of the milk.

Losses of moisture during curing. The effects of the delayed salting on moisture losses during the curing process are shown in table 5. There is normally a downward trend in the moisture content of Brick cheese during the first 14 days of curing. This is caused by evaporation losses and by the incorporation of salt. The salt has a double effect in that it tends to decrease the water-holding capacity of the curd and because, as it is absorbed, it increases the dry matter. The combination of these effects as shown in the data of table 5 indicates that the delayed salting of the curd increased the losses of moisture between making and paraffining at 14 days of age. After paraffining the losses were practically identical regardless of the previous

TABLE 4
Effect of delayed salting on the cheese acidity

Time of observation	Time of salting		
	1st day pH	5th day pH	9th day pH
Raw-milk cheese*			
Before salting	5.08	5.10
After salting	5.03	5.17
At 14 days	5.25	5.26
At 10 weeks	5.39	5.43
Pasteurized-milk cheese**			
Before salting	5.08	5.06	5.14
After salting	5.05	5.08	5.13
At 14 days	5.13	5.17	5.19
At 10 weeks	5.20	5.26	5.27

* Average values for 5 lots of raw-milk cheese.

** Average values for 3 lots of pasteurized-milk cheese.

salting treatment and regardless of whether the cheese was made from pasteurized or raw milk. It seems probable that the softening of the body,

TABLE 5
Effect of delayed salting on losses of moisture

Losses during the interval:—	Time of salting		
	1st day %	5th day %	9th day %
Raw-milk cheese*			
Before salting	0.0	1.2
During salting	2.6	2.2
Salting to 14 days	0.6	0.7
14 days to 10 weeks	0.6	0.6
Total moisture losses	3.8	4.7
Pasteurized-milk cheese**			
Before salting	0.0	0.8	1.1
During salting	1.6	2.3	1.7
Salting to 14 days	1.4	0.5	0.7
14 days to 10 weeks	0.9	1.0	0.9
Total moisture losses	3.9	4.6	4.4

* Average values for 5 lots of raw-milk cheese.

** Average values for 3 lots of pasteurized-milk cheese.

observed in the lots of cheese salted on the 5th and 9th days after making must therefore be attributed to protein changes rather than to the retention of more moisture.

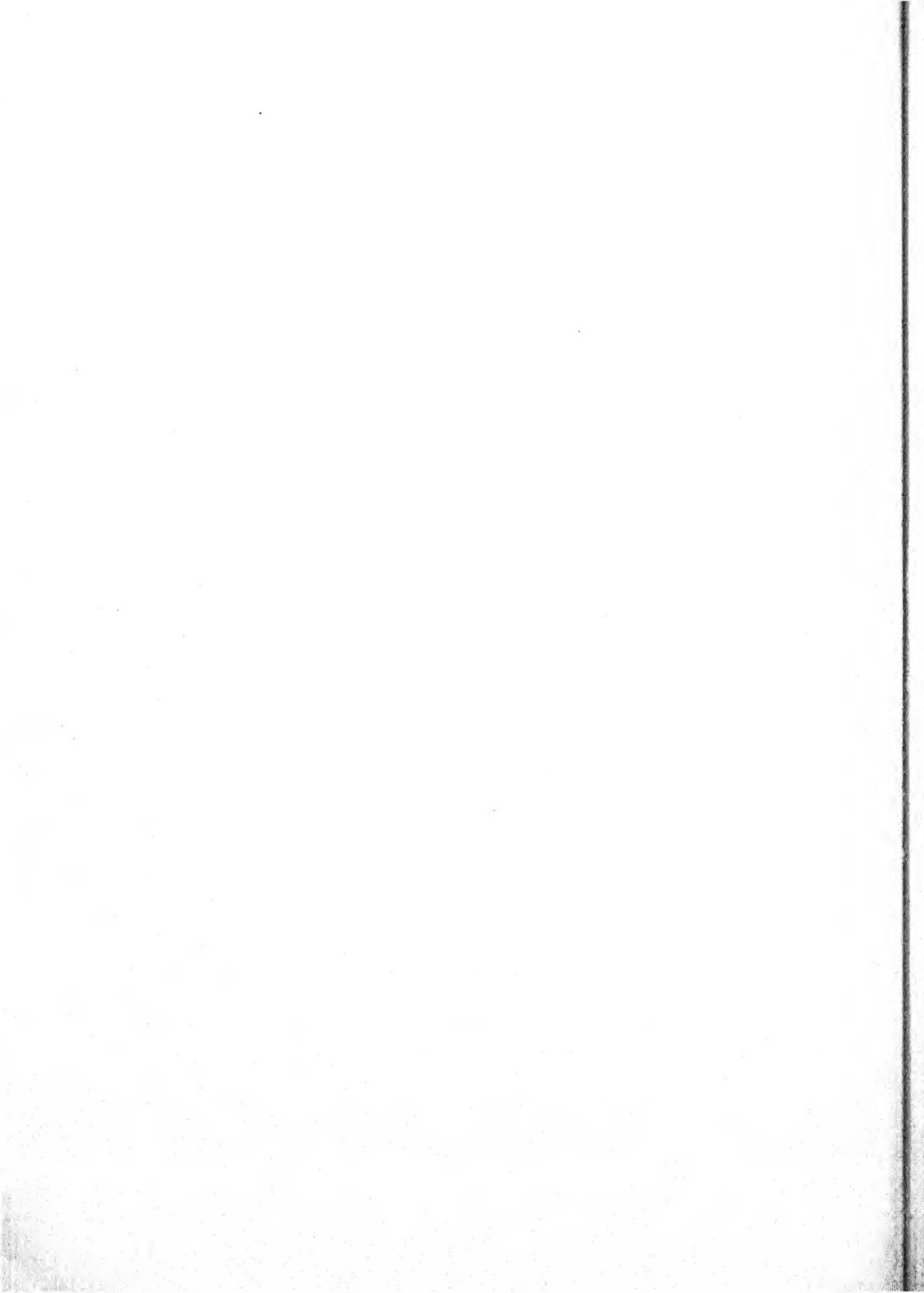
CONCLUSIONS

Delayed salting as practiced in these experiments seems to have no real benefits to commend it to the practical operator. There is an apparent improvement in the body of the cheese at the end of the 14-day period during which the cheese is retained in the factory. By the time the cheese has been cured, however, this benefit has disappeared and the general quality of the resulting cheese is not as good as that of the cheese salted in the normal manner. The addition of salt soon after making probably establishes a desirable trend in flavor production and body changes in Brick cheese curd that does not happen when salting is delayed.

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THE RELIABILITY OF THE ROOM TEMPERATURE HOLDING TEST AS AN INDEX TO THE KEEPING QUALITY OF BUTTER

D. H. JACOBSEN, C. C. TOTMAN AND T. A. EVANS

South Dakota State College, Brookings

The "room temperature holding test" is understood to include all butter keeping quality tests carried on by incubating small samples of butter for periods of from 6 to 10 days at temperatures of from 67° to 70° F. The actual practices in the various plants differ as to time and temperature but the general idea of predicting the keeping quality of butter in commercial channels on the basis of the flavor and odor developed at relatively high temperatures is common to all.

The need for such a test has been emphasized by a study made by Sprague, Foelsch and Small (1) of the butter offered on the large metropolitan retail markets. The survey was made of selected brands sold in one-pound cartons in New York and Chicago. In their conclusions the investigators stated that the instances in which deterioration had lowered the score more than one full point from the original score were few but still numerous enough to indicate that keeping quality is a serious problem. They state further that "For the purpose of identification of butter which lacks keeping quality and for the prevention of its use in cartons carrying certificates of quality, a wider use of incubation tests for keeping quality is desirable."

The relation of the time elapsed between grading and purchase and the loss in score showed that the time involved in the actual handling and sale of fresh butter supplies ranged up to 25 days, with an average of about 15 days. Since the butter was exposed to many temperature changes during transportation and finally in the retailers hands, the problem of stable butter quality was shown to be a very important factor in marketing.

PREVIOUS WORK

The prediction of the keeping quality of butter in ordinary commercial channels has been given increased attention in recent years probably due to the tendency toward lightly salted butter of higher flavor quality.

Hunziker (2) described two keeping quality or incubation tests involving holding small portions of butter at room temperature or higher. He stated that the tests are effective in revealing relative resistance of butter to flavor deterioration due to bacterial causes. He emphasized the use of the test in the prevention of surface taint by detecting fermentation in the small portions held in the test.

The value of the keeping quality test in the detection of faulty methods

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of production was indicated by Hammer (3). In a discussion of the development of methods of making keeping quality tests he emphasized the fact that the test probably can detect only those defects which are due to organisms rather than those due to chemical action. He states that "Temperature has a definite effect on the growth of organisms in butter, and a close correlation between the deterioration at various temperatures cannot be expected."

The use of a test involving parchment wrapped print butter was described by Parsons (4). The butter was held for fourteen days at 60° F. in a room of controlled humidity of 90 to 100 per cent. He found the test useful in the detection of butter of uncertain handling quality. He indicated that a test involving eight days at 70° F. gave equivalent results but that it required closer temperature control to prevent "oiling off" of the butter in certain seasons.

Sorensen (5) reported on an extensive survey of the keeping quality of both salted and unsalted butter in commercial channels by the use of the holding test. One-fourth pound parchment wrapped samples were held in a thermostatically controlled cabinet at 68° to 70° F. for seven days. The samples were examined for flavor and odor defects at the end of this period and reported as satisfactory, fair or unsatisfactory. A total of 22,060 churnings were examined representing more than 100 plants in eight midwestern states. The author states "a surprisingly close correlation between keeping quality tests and subsequent difficulty with the churnings tested was noted." The putrid-cheesy type of flavor defect was the most frequently encountered defect in the salted butter which showed unfavorable keeping quality. The value of the keeping quality test in locating contaminated water supplies or unsanitary plant conditions was pointed out.

Previous work at this station (6) has shown the relation between the numbers of bacteria in butter and the keeping quality at various temperatures. Similar high points in numbers of bacteria were developed in the incubation test for 7 days at 70° F., after 3 to 4 weeks at 40° F. and after 8 weeks at 32 to 36° F. The types of bacteria growing at these different temperatures varied and as might be expected the type of flavor varied with the holding temperature. It was found that lipolytic and proteolytic bacteria grew best at 40° F. as indicated by the fact that the counts reached higher levels at this temperature than at either the higher or lower temperatures. At 70° F. the lactic acid forming organisms usually predominated and their activity, no doubt, inhibited the growth of more objectionable types. The sour flavor developed at this temperature frequently was sufficient to mask other off flavors which were present and which would develop at lower temperatures.

THE PROBLEM

The holding test has been applied by many of the large butter manufacturers in recent years with good success. There has been a feeling on the

part of the operators, however, that the results of the keeping quality or incubation test has failed in certain cases to sort out properly all of the butter which was of uncertain quality. In some cases butter which failed to show definite deterioration in the incubation test would break down before it could reach the ultimate consumer while in other cases the deterioration developed in the incubation test was of a type which did not occur under the temperature conditions commonly found in butter warehouses and retailers' holding rooms. It was to obtain some information on possible causes of this lack of agreement that the following work was done.

PROCEDURE

The butter in this study was obtained from the educational scoring contests held at the station over a period of three years and included 78 lots representing 25 different creameries in South Dakota. The butter represented regular commercial churnings in some cases while in others it was made especially for the contests. All of the butter was salted with the salt content ranging from 0.5 per cent up to 3.0 per cent and averaging 1.8 per cent.

Samples were obtained with sterile spatulas and placed in 5-ounce glass jars with screw tops protected by parchment paper liners. Two samples were obtained from each tub, one to be held at 70° F. in a thermostatically controlled box, and one at 40° F. in the laboratory refrigerator. These lots were scored and examined for bacteria, yeast and mold when fresh and at intervals during the holding period. The scoring and microbiological analysis were done after 7 days at 70° F. and after 28 days at 40° F. All samples were tempered overnight to approximately 40° F. before scoring regardless of the temperature of holding to make the results of scoring more comparable.

The fresh butter scores were made by the official judges of the contests and by members of the department while the held butter was scored and criticized by members of the dairy department. The yeast and mold and bacteriological studies were made according to the methods suggested by the American Dairy Science Association Committee on Microbiological Analysis of Dairy Products (7).

RESULTS

In order to show the application of the Holding Test to the grading out or sorting out churnings of questionable keeping quality, the 78 lots were divided according to loss in score. In table 1 two methods of classifying the butter are presented. The average scores of the butter when fresh and the loss in score after holding are shown to permit a comparison of the keeping qualities of the two lots.

The results in table 1 under method A show the division of the butters into those showing less than one point loss in the holding test and those

TABLE 1

The comparative keeping quality of butter classified by the holding test

Method	Holding test Loss in score	Number of lots	Fresh score	Loss in score Holding test 7 days—70° F.	Loss in score 1 mo.—40° F.
A	less than 1 point	38	91.68	.10	.28
	1 point or more	40	92.36	2.12	1.44
B	1 point or less	55	91.92	.42	.58
	more than 1 point	23	92.32	2.80	1.91

showing one point or more loss. This method divided the 78 lots almost evenly. A slightly lower average fresh score was obtained in the class which showed less than one point loss. This might be expected because the deterioration in flavor score can be noted more easily when higher scoring butter is involved. The effectiveness of the holding test is indicated by the close relation between the holding test loss in score and the loss in score at 40° F.

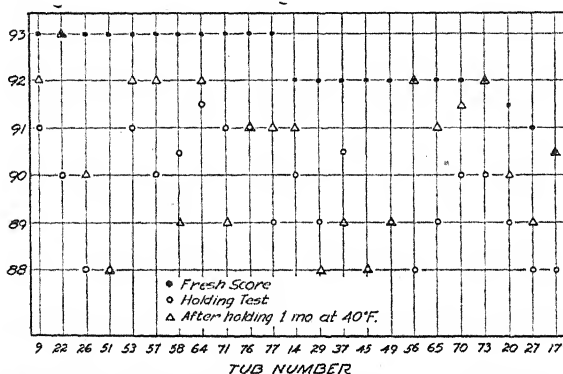


FIG. 1. Relation of "holding test" score to fresh score and to score after holding 1 month at 40° F.

(Lots losing more than 1 point in holding test)

Under method B in table 1 the butters were divided into two groups with only those showing more than one point loss being placed in the low keeping quality group. This division resulted in a smaller number of lots going into the low keeping quality group but the relation between groups was not greatly altered. The twenty-three lots which lost more than one point in the holding test were arranged in Fig. 1 according to the fresh butter scores to show the relationship between the holding test and the score after one month at 40° F. The results with individual lots are shown here to permit a study of the agreement between the test and the score of different lots after holding. In most cases the loss in score in the holding test and a loss during one month at 40° F. compare fairly well. In certain cases such as tubs num-

ber 22, 56, 73, and 17 losses in the holding test were not borne out in the butter held at the lower temperature. In three of these lots, cheesy or rancid flavors developed at room temperature but were not detected at the lower temperature within one month. A longer period at 40° F. might have brought out the flavor deterioration indicated in the holding test. As previously stated, however, it is probably more important to the butter manufacturer to know that the holding test finds the butters of uncertain keeping quality rather than to show absolute agreement with the record of each individual lot. It is apparent from these results that either method of classification results in a general segregation of those butters of low keeping quality from those of more satisfactory keeping quality.

There were exceptions to the general rule as indicated by the fact that seven of the fifty-five which lost one point or less in the holding test showed more than one point loss when held one month at 40° F. Six of these were given a score of 93 when fresh. Also eleven of the butters which lost more than one point in the holding test failed to show more than a point loss in the month storage at 40° F.

The value of the test in selecting butter of poor keeping quality is also indicated by a survey of individual lots in table 2 which showed that every lot which fell below 90 in score at the end of one month at 40° F. was classified in table 1 in the poor-keeping-quality group by the holding test. The greatest discrepancy between results appeared in those cases in which the holding test indicated poorer keeping quality than was found after holding at the lower temperature. Such lots as No. 17 and 56 which became cheesy at room temperature but held their original score at 40° F. were such cases.

The reason for such lack of agreement probably lies in the type of changes taking place in the butter at the different temperatures. The different types of flavors which developed under different holding conditions are shown in table 3. It may be noted that such flavors as feed, old cream and acid were much more prevalent in the fresh butter than after holding. Flavors such as stale, cheesy or rancid were most marked after the room temperatures holding test, while the flavors developed after one month at 40° F. were storage, coarse, stale, oily and rancid. These results indicate that the room temperature holding resulted in more bacterial deterioration than the lower temperature holding which permitted chemical action but limited the bacterial action. The difference in the flavors produced appears to indicate this conclusion.

The study of the numbers of lipolytic or fat splitting bacteria and proteolytic or casein digesting bacteria supported the viewpoint expressed above. These types of organisms were absent in most of the plates made from fresh butter but after 7 days at room temperatures, large numbers of proteolytic bacteria were sometimes found. The presence of these types was found to be associated with the development of cheesy flavor in most cases. At the

TABLE 2

The "Holding Test" and loss in score after one month at 40° F.

Tub number	Fresh butter		Holding test 7 days at 70° F.		Held one month at 40° F.	
	Score	Criticism	Score	Criticism	Score	Criticism
1	92	old cream	92	sl storage	92	sl storage
2	92	old cream	92	coarse	91	stale
3	92	sl oily	91	sl stale	91	stale, briny
4	93		92	flat	92	
5	93		92	chem	92.5	
6	92	sl feed	92	briny	92	briny
7	93	sl bitter	92	flat, briny	91	stale
8	91.5	old cream	92		91.5	sl stale
9	93		91	tallowy	92	
10	92.5	sl bitter	92	coarse	92	coarse
11	91	bitter	91	tallowy	91	sl stale
12	93		93		92	sl stale
13	93		92	sl stale	92	sl stale
14	91.5	old cream	90	sl cheesy	91	stale
15	92	old cream	92	sl stale	91.5	sl storage
16	91	briny, old cream	91	coarse	91	coarse
17	90.5	briny, old cream	88	sl cheesy	90.5	old cream
18	93		93		93	
19	93		92	sl acidy	93	
20	91.5	stale cream	89	unclean	90	stale
21	89	neutralizer	89	storage	89	storage
22	93	acidy	90	sl rancid	93	
23	93		93		93	
24	90	neut. weedy	89	stale	90	briny, weedy
25	91	neut. coarse	91	coarse	91	briny, grassy
26	93		87	rancid	90	flat, sl oily
27	91	cooked, sl uncl	88	unclean, acidy	89	unclean
28	93		93		91	storage
29	92	flat, sl old cream	89	moldy	88	sl rancid
30	93		92.5	flat	93	flat
31	93		92	sl storage	92	
32	91.5	old cream	92	coarse	91.5	storage
33	91	sl unclean	90	stale, sl rancid	90	storage
34	92	sl coarse, burnt	92.5		91.5	sl malty
35	91.5	acidy	91.5	acidy	91	briny, acidy
36	92	briny, acidy	91.5	briny	92	
37	92	sl acidy	90.5	acidy, stale	89	sl moldy
38	91	acidy	92	coarse	91	coarse
39	92	sl flat	93		92	
40	93		93		91	stale
41	92	briny, acidy	92.5		91	coarse
42	90	briny, metallic	91	briny	91.5	briny
43	92	acidy	91.5	storage	92	
44	92	acidy	92	acidy	91	sl flat
45	92	cooked	88	rancid	88	stale, fishy
46	91	burnt	91.5	stale	91	coarse
47	91	sl unclean	91	sl unclean	91	sl stale
48	92	sl acidy	92		91.5	briny
49	92	acidy	89	oily, acidy	89	stale
50	92	sl utensil	91	flat	93	
51	93		88	cheesy, oily	88	fruity, rancid
52	92.5	sl barny	92.5		92	coarse
53	93		91	sl fruity	92	sl stale
54	92	sl feed	91	sl unclean	92	

TABLE 2—(Continued)

Tub number	Fresh butter		Holding test 7 days at 70° F.		Held one month at 40° F.	
	Score	Criticism	Score	Criticism	Score	Criticism
55	93		92	flat, sl tallow	93	flat
56	92	utensil	88	cheesy, rancid	92	
57	93		90	stale	92	
58	93		90.5	bitter, metallic	89	rancid
59	90.5	stale, malty	92	flat	92	
60	90	met., burnt, neut.	91	sl tallow	91	old cream
61	93		92	sl storage	92	sl storage
62	93		93		93	sl coarse
63	91.5	sl musty	91	sl stale	90	oily, stale
64	93		91.5	sl stale	92	sl storage
65	92	wintery	89	sl cheesy	91	stale
66	93		92		91.5	coarse
67	90	burnt, metallic	92	coarse	91	briny, old cream
68	93	heated	92		90	oily, neut.
69	92	briny	91	sl stale	91	storage
70	92	sl coarse, feed	90	sl cheesy	91.5	coarse
71	93	sl heated	91	sl stale	89	woody
72	91	burnt, malty	92	sl bitter	91	burnt, old cream
73	92	coarse, briny	90	stale	92	coarse
74	93		92		91	flat, oily
75	91	malty	92	feed	92	briny
76	93	sl feed	91	sl stale	91	feed, stale
77	93		89	cheesy	91	woody
78	92	stale	91	sl stale	92	sl stale

TABLE 3

Flavor criticisms used in scoring butter

Flavor criticism	Fresh score	Holding test 7 days—70° F.	Held 1 month 40° F.
Number of lots scored	78	78	78
No criticisms	20	16	15
slight old cream or old cream	8	0	3
slight acid, acid or coarse	14	13	11
briny	6	4	9
slight feed, feed or weedy	5	1	1
neutralizer	3	0	1
slight unclean or unclean	3	4	1
Cooked, heated	4	0	0
tallowy	0	4	0
slight storage or storage	0	5	9
slight stale or stale	2	15	18
slight cheesy, cheesy or fruity	0	8	1
utensil	4	0	0
slight rancid or rancid	0	5	3
oily, stale	1	2	4
malty	3	0	0
moldy	0	1	1
burnt	4	0	0
fishy	0	0	1
bitter	3	1	0
flat	3	2	0
woody	0	0	2
metallic	3	1	0

lower temperature of 40° F. there was very little evidence of bacterial activity which could be directly associated with flavor deterioration although large numbers of proteolytic bacteria were occasionally found after one month at 40° F. Lipolytic bacteria were absent except in a few lots in which small numbers, usually less than 1000 per ml. were found by the plate method.

Yeast and mold counts on the fresh butter failed to show any relation to the keeping quality of the butter in these trials. This is in agreement with the statements of numerous investigators working on this problem. The number of yeasts was generally high in the butter held at room temperature for 7 days but no correlation with the flavor deterioration could be noted.

CONCLUSIONS

In conclusion, these results indicate that the holding test is useful and fairly accurate as a means of detecting butter of unstable handling quality. The chief factor influencing the reliability of the test appears to be the difference in activity of certain types of bacteria at the incubation temperatures and at lower temperatures.

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A NEW DILUENT FOR BOVINE SEMEN

C. E. KNOOP

Ohio Agricultural Experiment Station, Wooster

A desirable diluent for semen is one that will maintain fecundity of the spermatozoa for many days before it is used for breeding purposes.

Considerable experimental work with diluents versus no diluents for semen has been done in Europe and the United States. The use of egg yolk lecithin in a diluent for semen by Milovanov and Selivanova (2) in Russia, and the use of egg yolk by Phillips and Lardy (4) of Wisconsin has proved helpful in keeping sperm cells viable for some time. The work of Milovanov (2, 3) and Phillips and Lardy (4) suggested an investigation of a combination of gelatin, egg yolk, buffer salts and water, as a diluent material in the artificial insemination studies in progress at the Ohio Agricultural Experiment Station. It is believed that gelatin (Knox) tends to hold sperm inactive, assists in keeping the particles of the egg yolk and the sperm in suspension, supplies extra nutrients, and retards general contamination (bacteria and molds) during storage.

Preliminary work done at this station with the gelatin and the non-gelatin diluents has given encouraging results in favor of the gelatin. Two series of samples have been studied. In the first series 12 samples of bovine semen were diluted four times with a diluent containing 2.14 gm. of gelatin (Knox), 0.2 gm. of KH_2PO_4 , 1.325 gm. of $\text{Na}_2\text{HPO}_4 \cdot 12 \text{H}_2\text{O}$, 100 cc. of sterilized distilled water, and 100 cc. of fresh egg yolk. The non-gelatin group consisting of five samples of bovine semen were diluted four times with a diluent containing 0.2 gm. of KH_2PO_4 , 1.325 gm. of $\text{Na}_2\text{HPO}_4 \cdot 12 \text{H}_2\text{O}$, 100 cc. of sterilized distilled water, and 100 cc. of fresh egg yolk. The materials other than the egg yolk of both diluents were first dissolved in the water before adding the egg yolk. The range in pH was from 6.7 to 6.85. The vials containing the diluted samples were wrapped in cotton and placed in a refrigerator maintained at 4° to 6° C. Periodical examinations were made with a microscope after a drop of the diluted semen was placed on a glass slide and warmed to 37° C. The gelatin dilutions maintained some sperm motility for an average of 21½ days (range 18 to 30 days), whereas the non-gelatin mixture maintained some sperm life for an average of 14½ days (range 14 to 16 days).

The technique of handling the semen in the second series was as follows: Collection and dilution of the semen was carried out under sanitary conditions; diluted samples were gradually cooled from 30° to 5° C. for storage purposes; and periodical examinations were made of the stored samples with a compound microscope ($\times 440$) after a small portion was rediluted and

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gradually warmed from 5° to 37° C. The gelatin and non-gelatin diluents were the same as those used in the first series, except that different amounts of gelatin and $\text{Na}_2\text{HPO}_4 \cdot 12 \text{H}_2\text{O}$ were used from time to time. The range in pH was from 6.45 to 6.9. The results are given in table 1.

TABLE 1

The effect of gelatin and no gelatin in a diluent upon motility of bovine spermatozoa in storage

Diluent contains	Number of samples	Per cent of sperm motile after 2 to 4 days	Average number of days when		
			50 per cent motility was observed	25 per cent motility was observed	All cells were dead
Gelatin (Knox)	26	73 (57-83)*	12.5	17.5	26.5 (16-35)*
No gelatin	14	59 (33-80)*	8.3	14.5	26.0 (14-38)*

* Figures in parentheses represent range.

Maintaining motility in 50 per cent of the spermatozoa an average of four days longer than previously possible and keeping a few alive for 35 to 38 days is stimulating to future work.

According to the literature the previous record for keeping motile bovine sperm in a diluent following collection seems to be 12.5 to 13 days (1, 4).

Artificial breeding of a large number of cows with semen that has been diluted with these two diluents is now in progress.

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SOME OCULAR CHANGES AND DEFICIENCY MANIFESTATIONS IN MATURE COWS FED A RATION DEFICIENT IN VITAMIN A*

L. A. MOORE¹

Dairy Department, Michigan Agricultural Experiment Station, East Lansing

In previous publications (1, 2, 3) a type of blindness has been described which occurred in calves fed low vitamin A rations. The blindness was associated with a constriction of the optic nerve, nyctalopia, and papilledema. The cause of the papilledema was later established as directly due to an increased intracranial pressure (4) in vitamin A deficiency. This type of blindness has never been reported as occurring in the mature bovine because, as previously explained (2, 3), the optic foramina are fully developed and calcified. However, papilledema and nyctalopia develop as well as certain other ocular changes. It is the purpose of this paper to report the ocular changes and deficiency manifestations where the mature bovine was fed a ration deficient in vitamin A.

EXPERIMENTAL

Mature cows were used in this experimental work. They were placed on the low carotene ration previously used with calves which consisted of, 36.0 per cent barley, 27.0 per cent rolled oats, 27.0 per cent wheat bran, 9.0 per cent linseed oil meal, and 1.0 per cent salt. This ration contained from 0.5 to 0.7 micrograms of carotene per gram so that the animals received 2 to 3 micrograms per pound of body weight from this source. Viosterol and sunshine were used as sources of vitamin D. Wood shavings were used as bedding.

Ophthalmoscopic observations were made at various intervals and the animals were tested for night blindness by attempting to run them into objects, and watching their behavior in dim light, a method similar to that used by Guilbert and Hart (5). Blood plasma carotene determinations were made at intervals by a method previously described (6). Carotene extractions on the hays and feeds used were made according to the modification by Peterson *et al.* (7) of the Guilbert method and the concentration of the extract determined by a photoelectric colorimeter.

Before proceeding further it would probably be of assistance to the reader to explain the first three figures. Figure 1 shows the normal bovine fundus. The nerve head or papilla is seen in the center; the tapetum lucidum which consists of the upper yellow part of the retina and the

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¹ Now at the University of Maryland.

tapetum nigrum the lower dark part of the retina are seen surrounding the papilla. There are of course individual variations of the normal fundus in the outline, color and distinctness of the nerve head, the color of the tapetum lucidum arrangement of the vessels, etc. The tapetum nigrum is always of a dark shade. The color of the tapetum lucidum is affected by the amount of exposure to bright light. The normal yellow color as shown in figure 1 will become bleached after the animal has been exposed to bright light such as the sun. These figures were made from animals not exposed to bright light.

Figure 2 shows the mottled appearance of the tapetum nigrum. The tapetum lucidum is bleached and the nerve head shows a cottony white appearance, evidence of papilledema.

Figure 3 shows the mottled appearance of the tapetum lucidum and papilledema. The mottled condition as illustrated in figures 2 and 3 occurs only in the more mature animal or after about 18 months of age.

A1 was a 5-year-old grade Holstein cow which had been receiving a ration in which the sole source of vitamin A was yellow corn. This ration, while apparently adequate for maintenance, had not contained sufficient vitamin A for proper reproduction. The vitamin A reserve for this animal was therefore probably much less than for an animal which had been receiving hay. Further, she had been milking on this ration up to the time she was placed on this experiment. The eyes were normal when placed on the low vitamin A ration except for a narrow violaceous area, $1\frac{1}{2}$ cm. in length, along the temporal vessels.

After 18 days on the low vitamin A ration there was definite nyctalopia, the nerve heads of both eyes were somewhat hazy in appearance, and the tapetum nigrum showed some slight mottling as illustrated in figure 2. By 53 days the margins of the nerve head were definitely indistinct, but not markedly edematous, the mottling of the tapetum nigrum had increased, and the tapetum lucidum was somewhat bleached. At 81 days alfalfa was added, supplying 14 micrograms of carotene per pound of body weight. At 116 days this cow was no longer night-blind and the plasma carotene had increased from 0.18 to 0.4 micrograms per milliliter. The alfalfa was eliminated from the ration at 133 days and at this time the animal got out and obtained a good fill of green grass so that the plasma carotene increased from 0.4 up to 0.9 micrograms per milliliter. This accounts for the relatively long time it took to develop nyctalopia again.

At 236 days this animal again became nyctalopic and the edema of the nerve heads increased so that there was a choking of 2 diopters in each eye. At 249 days carotene was again added in the form of alfalfa at the rate of 14 micrograms per pound of body weight and at 269 days she was no longer night-blind. At 291 days both nerve heads were still edematous but the mottled appearance of the tapetum nigrum had quite largely disappeared.

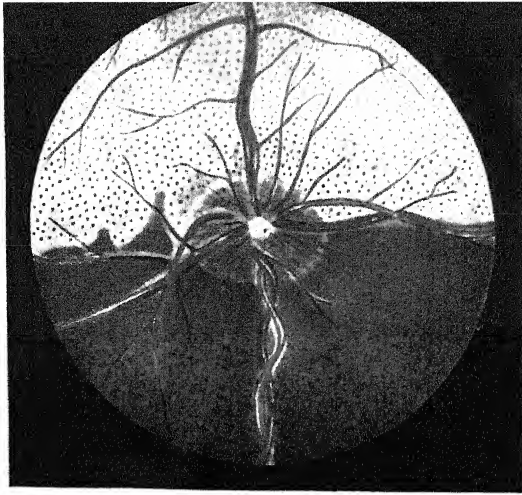


FIG. 1. Normal bovine fundus.

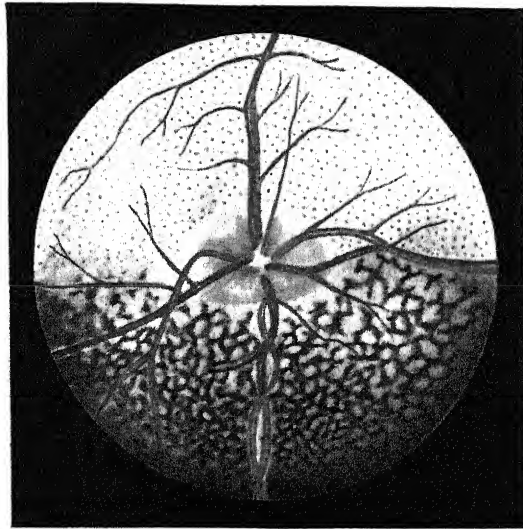
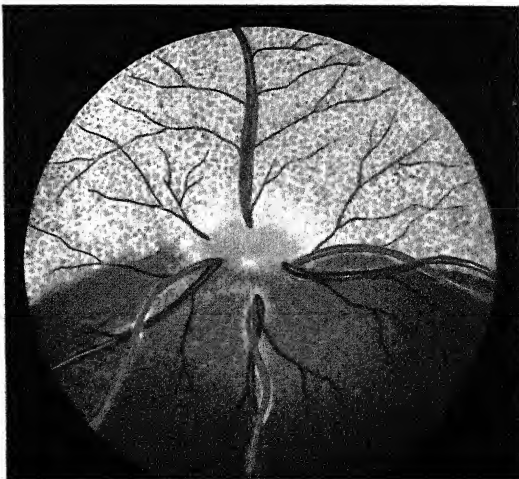
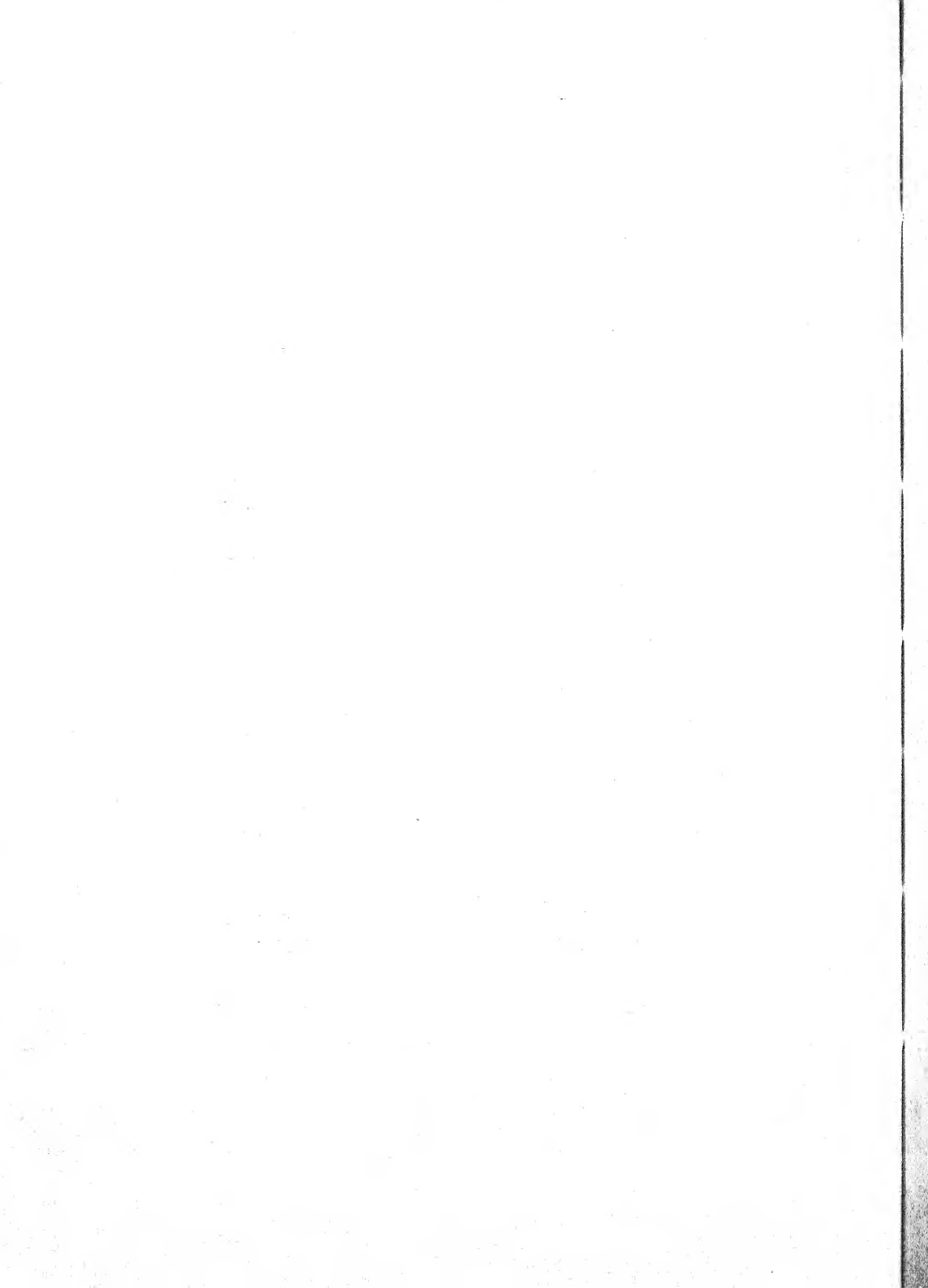


FIG. 2. Fundus showing papilledema, bleached tapetum lucidum and a mottled tapetum nigrum.



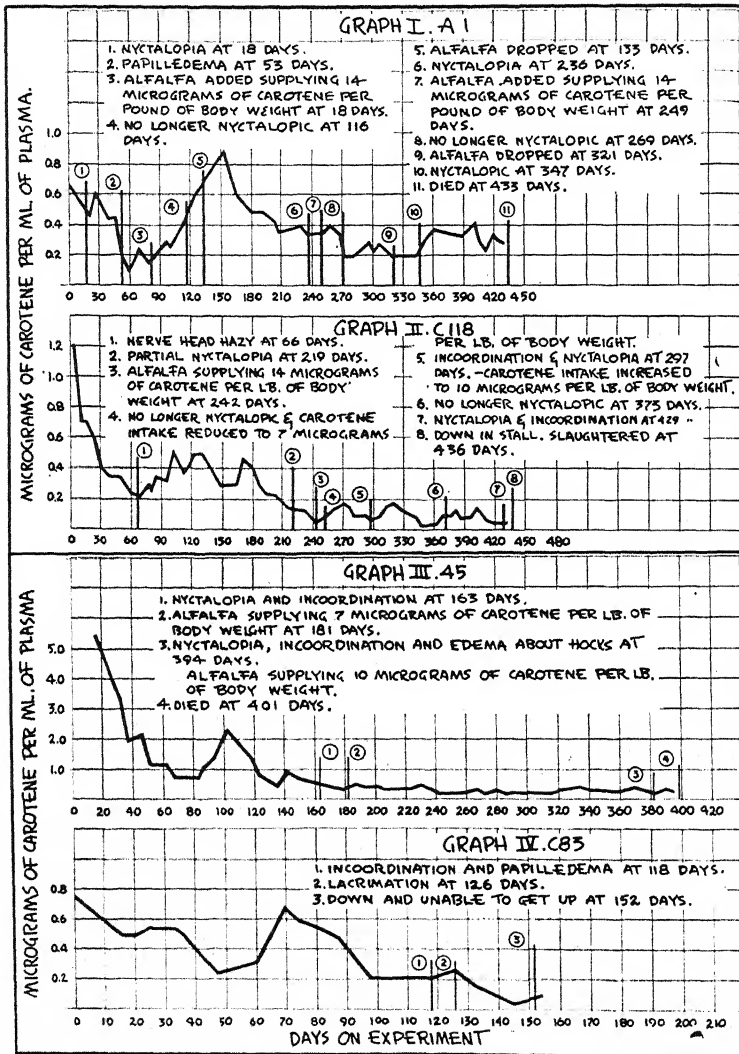


At 321 days the alfalfa was again eliminated from the ration. At 347 days she was again night-blind and by 421 days the edema had increased so that there was a choking of 3 diopters in both eyes and marked mottling of the tapetum nigrum. The cornea was slightly opaque and there was considerable lacrimation. At 411 days she was quite weak, showed marked incoordination and died at 433 days. The principal results along with the variations in level of blood plasma carotene are shown in graph I. Post-mortem examination revealed considerable pneumonia and other lesions associated with vitamin A deficiency which will be reported in a subsequent paper.

Cow C118 was a six-and-one-half-year-old Holstein cow which had been on a grain ration containing corn gluten and yellow corn as a source of vitamin A. This cow had been dry for a considerable period and calved about three weeks before being placed on the low vitamin A ration. This animal was dried up about three weeks after being placed on the low vitamin A ration so that she was milked for only about six weeks. Consequently, she probably had some storage when placed on the low carotene ration. The principal results are shown graphically in graph II.

Twenty-nine days after being placed on the low carotene ration, the tapetum nigrum of each eye showed definite mottling. At 66 days the papillae were quite hazy in appearance, but showed no elevation. At 95 days both nerve heads were hazy, but the margins could still be seen and there was no apparent elevation. She did not appear quite so active. At this time C118 got out of the pen at night and obtained some green material which probably delayed somewhat the later changes. At 165 days the tapetum lucidum was bleached and the tapetum nigrum quite mottled. At 219 days there was some indication of nyctalopia. At 230 days the animal showed considerable incoordination and a poor appetite. The nerve heads showed slight edema, but the margins were still discernible. There appeared to be little or no elevation. Alfalfa was added at 242 days to supply 14 micrograms of carotene per pound of body weight because of the extreme incoordination. At 262 days she was no longer night-blind, and the alfalfa intake was reduced to supply 7 micrograms of carotene per pound of body weight. At 276 days she no longer showed indications of incoordination and was fairly active although there was some edema in the rear legs. At 297 days she again manifested night-blindness, incoordination and had a rough appearance. At this time, the alfalfa was increased to 10 micrograms of carotene per pound of body weight. The incoordination largely disappeared at this level of intake but she remained partially nyctalopic till the 373rd day. The plasma carotene, however, remained exceedingly low at this level, and at 429 days nyctalopia and incoordination were again manifested. At 433 days the nerve head showed some edema, but the margins were still discernible. There was marked mottling of the tapetum nigrum and slight mottling of the lucidum as shown in figure 3. The tapetum lucidum was also

bleached. At 436 days the animal was quite weak, showed marked incoordination, got down in her stall and was unable to get up. The next day she was slaughtered in order to save the tissue for pathological study.



Cow 45 was a three-and-a-half-year-old Guernsey cow which had received a normal ration. She was placed on the low carotene ration the middle of June and had received some pasture so that the plasma carotene was quite high. After 163 days on the low carotene ration this animal showed nyctalopia, a slight bleaching of the tapetum lucidum, and was unsteady on her feet. The carotene in the blood plasma had decreased to 0.5 micrograms per

milliliter at this time as shown in graph III. After 172 days there was some yellowish discoloration just dorsal to the nerve heads. At 181 days alfalfa leaf meal was added to supply 7 micrograms of carotene per pound of body weight or one-half the minimum requirement. At this level she continued to show considerable incoordination and remained nyctalopic. At 349 days the tapetum lucidum was bleached somewhat but the nerve heads showed no evidence of papilledema. At 381 days the animal was unsteady on her feet, and there was considerable edema about the feet and legs. The carotene intake from the hay was then raised to 10 micrograms per pound of body weight.

At 394 days the tapetum lucidum of both eyes was entirely bleached and she walked slowly because of the edema about the feet. She died after 401 days on the low carotene ration without showing evidence of papilledema.

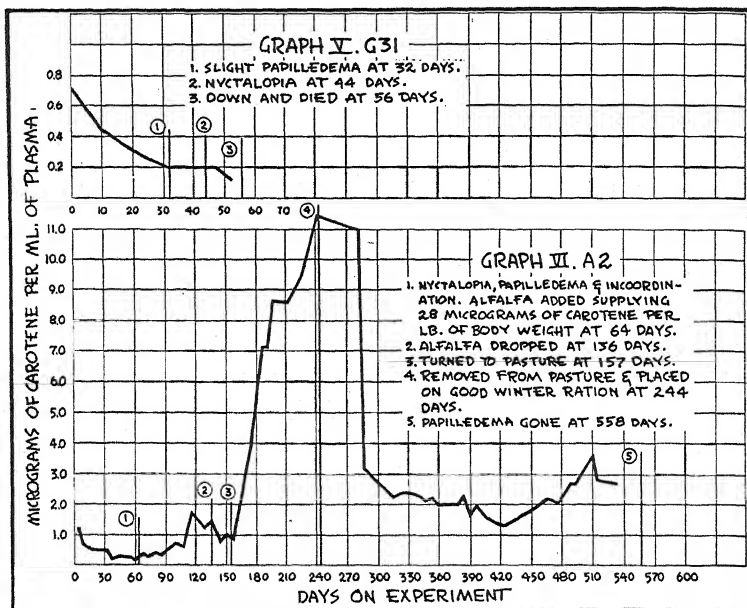
At post-mortem, aside from the changes due to vitamin A deficiency, the most notable phenomenon was the yellow color of the fat in the various parts of the body. A sample of the fat was weighed out, saponified with alcoholic KOH and extracted with petroleum ether. The petroleum ether was extracted with 92 per cent methyl alcohol to remove any xanthophylls. The extract showed a carotene content of 19 micrograms per gram. Further, this animal was in good condition and showed considerable deposition of fat in the mesentery. Post-mortem examination also showed the presence of pneumonia.

Cow C83 was an exceptionally fat seven-year-old Holstein which had previously been receiving a ration of skimmed milk, yellow corn, oats and viosterol. The ration had always been kept rather low in vitamin A during most of this animal's life. At the time this cow was placed on the deficient ration no routine ophthalmoscopic examinations were being made, nor was she tested for night blindness.

After 118 days on the low carotene ration marked incoordination was noted and examination of the eyes revealed an extreme papilledema. At 126 days excessive lacrimation was noted. At 145 days the appetite was poor and the plasma carotene had decreased to a 0.05 level as illustrated in graph IV. At 152 days she got down and was unable to get up. It was necessary to sacrifice the animal two days later because she could not get into position to eat.

G31 was a seven-year-old cow which had previously been receiving a ration in which the sole source of vitamin A was yellow corn. She was in excellent condition when placed on the low carotene ration and the eyes appeared normal. Thirty-two days after being placed on the low carotene ration the nerve heads were blurred in appearance. There was one diopter of papillary edema at 44 days in the right eye and 2 diopters in the left and the nerve head showed considerable vascularity. At this time she was also nyctalopic and walked rather slowly and stiffly. At 52 days there were 2

diopeters choking in each eye. At this time she got down in the yard and was unable to get up alone. At 56 days she got down in the stall and was unable to get up. She was removed to a box stall and carotene in oil and linseed meal was administered by stomach tube. Cod liver oil was also given subcutaneously. She never regained her feet, however, and died during the night of tympanites. These observations as correlated with the level of blood plasma carotene are shown in graph V.



A2 was a five-year-old Holstein cow and had been receiving a grain mixture containing yellow corn as the only source of vitamin A. Ophthalmoscopic observations were not made on this animal until 64 days after she had been placed on the low carotene ration. At this time there was a choking of 2 diopters in the right eye and 4 diopters in the left and the nerve margins were entirely covered with the edema. She also showed considerable incoordination at this time and there was some suspicion that she was night-blind. The plasma carotene had decreased to 0.15 micrograms per milliliter. At this time alfalfa supplying 28 micrograms of carotene per pound of body weight was added to the ration. The alfalfa was dropped from the ration at 136 days. At 157 days the eye conditions remained the same and she was turned to pasture for the purpose of noting how long it would take these changes to clear up. It will be noted that the plasma carotene increased with this change as shown in graph VI. At 244 days she was removed from pasture and placed on a ration of alfalfa hay and corn silage which was followed by a rapid decline in plasma carotene. By 273 days the edema of

the nerve head had receded some and the nerve fibers could be seen.¹ At 341 days the nerve head had lost most of the cottony white color associated with the edema and had taken on a darker color which was more nearly normal. By 422 days the right eye was about normal but the margins of the left eye were still somewhat indistinct. By 558 days no edema could be seen in either eye and the nerve margins were fairly distinct. The nerve head, however, was still somewhat elevated since the readings were one diopter in the right eye and 2 diopters in the left. This was probably a residual effect of the long continued papilledema.

DISCUSSION

The results obtained with the animals of this experiment show that none developed the permanent type of blindness due to constriction of the optic nerve such as occurs in calves (1, 2, 3) on vitamin A deficient rations. Several of these animals were permitted to develop extreme deficiency symptoms yet never developed the blindness. Insofar as the author is aware, blindness has never been reported as developing in a mature bovine due to a constriction of the optic nerve associated with vitamin A deficiency. The explanation of this observation as previously set forth (2, 3) appears to be due to the fact the bony optic canal grows in length from one-fourth inch in a young calf to about one and a half inches in a mature animal. In vitamin A deficiency in a calf the normal growth processes are affected in such a manner as to cause a stenosis of the bony canal with a consequent constriction of the optic nerve (1, 2, 3). Wolbach and Bessey (8) have noted an overgrowth of the central nervous system in vitamin A deficiency in young rats. They noted the presence of herniations of the cerebrum, cerebellum and posterior colliculus with changes in the contours of the fossae of the floor of the skull due to bone resorption. If such an overgrowth of the central nervous system takes place in the bovine species it could easily account for the increased intracranial pressure reported from this station (4).

The results likewise show that papilledema does not develop as readily in mature animals as in young calves. Animal 45 did not develop evidence of papilledema while the nerve head of C118 showed only a slight hazy appearance. Usually the papilledema did not develop until considerable incoordination was present. Unpublished data indicate that the differences are probably explained by individual and age variations of intraocular tension. It would seem that a higher intracranial pressure would be necessary to overcome a high intraocular tension than a low one in order to permit the development of papilledema.

Besides the presence of papilledema in cows fed vitamin A deficient

¹ Papillary edema existing for any length of time results in secondary atrophy (post-papillitic atrophy).

rations, certain other changes were observed with the ophthalmoscope. These consisted of a mottled appearance of the tapetum nigrum and occasionally a mottled appearance of the tapetum lucidum. These two alterations are illustrated in figures 2 and 3 and may be compared with the normal fundus shown in figure 1. Both these conditions were cleared up by administration of some source of vitamin A. Usually the changes were more easily observed and were more marked in the nigrum than in the lucidum. The micropathological alterations of the retina associated with these changes have not been investigated.

The papilledema of animal A2 took considerable time to recede even though she was turned to pasture or kept on good winter feed. The observation was duplicated in another animal not considered in this report. In older calves the same was true but not to such an extent. It has also been noted in calves that the intracranial pressure takes considerable time to return to normal after the return of carotene to the ration (4).

It is interesting to note that animal 45, a Guernsey, had a very yellow fat at autopsy. The pigment was epiphasic between petroleum ether and 92 per cent methyl alcohol so that it was most likely carotene. These results seem paradoxical in view of the fact that the animal showed marked symptoms of vitamin A deficiency. One must conclude that the animal was unable to draw extensively from this store of carotene.

Another interesting observation was that the more flesh the animal carried at the time the deficiency started to show up, the quicker the animal succumbed to the deficiency. Warm weather also seemed to be hard on the deficient cows. C83 and G31 were in exceptionally good flesh and were able to withstand the effects of the deficiency for only a short time. On the other hand C118 and A1 which were poor when the deficiency symptoms were first observed seemed to withstand the deficiency much better. C83 and G31 both got down in the stall and were unable to raise their heads. It is thought that this acute condition is due in part to an abnormally high intracranial pressure. In another experiment the intracranial pressure of a young male on the deficient ration was found to be equal to 500 millimeters of saline while the normal is about 100 millimeters. This animal was just able to get up and draining the spinal fluid gave a short period of relief.

In calves when the level of plasma carotene had decreased to about 0.13 micrograms per milliliter nyctalopia and papilledema and other evidences of vitamin A deficiency began to appear (9). In mature cows this level would appear to be somewhat higher. Animal 45 showed evidence of nyctalopia at 0.5 micrograms level so that a range of 0.2 to 0.5 level should be considered. Davis and Madsen (10) reported a level of 0.25 for heifers of the Shorthorn and Hereford breeds.

From the results obtained with animals 45 and C118 it would appear that an intake of 9 to 12 micrograms of carotene per pound of body weight was

not sufficient to prevent the development of symptoms of vitamin A deficiency. This intake was made up of the carotene fed in the alfalfa meal at the rate of 7 to 10 micrograms and the 2 to 3 micrograms present in the basal ration per pound of body weight. A total intake of 16 micrograms, however, appeared to be sufficient as shown by the results for A1 and C118. These observations agree with our previous observations (9) and those of Guilbert and Hart (5) who found that an intake of about 30 micrograms per kilo was necessary to prevent nyctalopia. However, it seems questionable whether this intake is the physiological minimum as stated by Guilbert and Hart since it is not sufficient for proper reproduction, or to prevent the development of an increased intracranial pressure in calves as shown by unpublished data from this station.

It will be noted in this paper that no cases of xerophthalmia are recorded even though extreme vitamin A deficiency was permitted to develop. In some cases considerable lacrimation and some clouding of the cornea were noted. The absence of xerophthalmia was confusing in the early work at this station on vitamin A deficiency and led to considerable doubt as to whether the deficiency seen was actually due to lack of vitamin A (1). However, the explanation of the apparent discrepancy probably lies in the environmental conditions under which these experiments were conducted. The eyes of the animals during the deficiency period were probably not subjected to the presence of large amounts of abrasive dust particles and possibly the proper type of bacteria.

SUMMARY

1. Mature cows on a vitamin A deficient ration failed to develop blindness due to constriction of the optic nerve such as has been reported in calves.
2. A definite papilledema failed to develop in two cows out of six in these experiments. Once the papilledema develops it takes considerable time for it to recede.
3. Mature cows did develop nyctalopia, incoordination, and an edema of the legs on the A deficient ration.
4. The tapetum nigrum and lucidum developed a mottled appearance.
5. When the plasma carotene values receded to a 0.2 to 0.5 microgram level deficiency symptoms usually followed in a short period of time.
6. The fat of a Guernsey cow which died with symptoms of vitamin A deficiency showed the presence of a pigment which was most likely carotene since it was epiphasic between petroleum ether and 92 per cent methyl alcohol.

The author wishes to express his appreciation to Dr. J. O. Wetzel of Lansing for his criticisms in preparing this paper.

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Announcement

Translations of a number of Danish and Swedish articles of interest to readers of the JOURNAL OF DAIRY SCIENCE have been completed in a W.P.A. project sponsored by the University of Minnesota. These translators assigned to W.P.A. Official Project No. 65-1-71-140, Sub-Project No. 484, have been supervised by Dr. Harold Macy. Copies of these translations are available in the Office of the American Documentation Society, 2101 Constitution Avenue, Washington, D. C.

Translations of the following Danish dairy articles are now available.

Smørrets Vandindhold og Saltning (Water content and salting of butter). H. Hendemann. 15^{de} Beretning fra Statens Forsøgsmejeri, Hillerød, Denmark. 1938.

Forsøg med Silkeborg Stassano apparat Model 1937. (Experiments with Silkeborg Stassano apparatus, Model 1937). Joho. Jensen and others and Sv. Horning. 16^{de} Beretning fra Statens Forsøgsmejeri, Hillerød, Denmark. 1938.

Forsøg med "Spirala" til Varmebehandling af Konsummaelk. (Experiments with "Spirala" for heat-treatment of consumers' milk). H. Jörgensen. 17^{de} Beretning fra Statens Forsøgsmejeri, Hillerød, Denmark. 1939.

Forsøg med "A.P.V." Apparat til Varmebehandling af Konsummaelk. (Experiments with the "A.P.V." apparatus for heat-treatment of consumers' milk). A. Petersen and K. Rasmussen. 18^{de} Beretning fra Statens Forsøgsmejeri, Hillerød, Denmark. 1939.

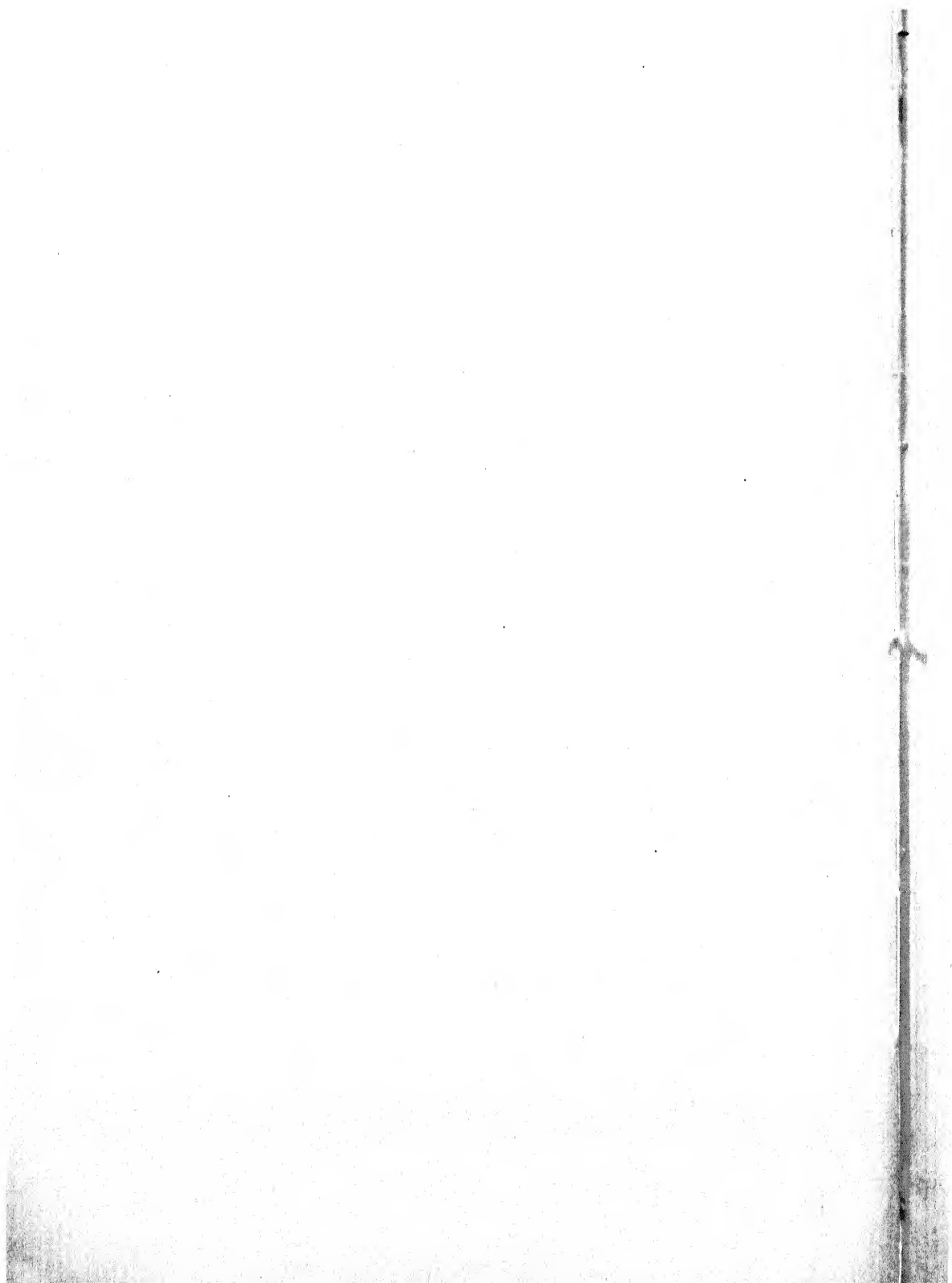
Forsøg med Pladeapparat "Kolding" Type B.P.K. til Varmebehandling af Konsummælk. (Experiments with the plate apparatus "Kolding," type B.P.K. for heat treatment of consumers' milk.) N. Kjærgaard-Jensen. 20^{de} Beretning fra Statens Forsøgsmejeri, Hillerød, Denmark. pp. 1-26. 1939.

Af prøvning af Victoria-Kubus Kaerneaelter. (Testing of the Victoria-Kubus churn-worker.) N. Kjærgaard-Jensen. 22^{de} Beretning fra Statens Forsøgsmejeri, Hillerød, Denmark. pp. 1-21. 1939.

Forsøg med Victoria-Kubus Kaerneaelter. (Experiments with the Victoria-Kubus churn-worker.) N. Kjærgaard-Jensen. 24^{de} Beretning fra Statens Forsøgsmejeri, Hillerød, Denmark. pp. 1-28. 1939.

Translation of the following Swedish article is also available.

Inverkan av vissa Konserveringsmedel på Mögel—och Jastsvampar från Ost. (Effect of certain preservatives on moulds and yeasts from cheese.) K. E. Thome. Meddeland No. 3 från Statens Mejeriförsök Särtryck ur Arsskrift för Alnarps lant-bruksmejeri-och trödgårds-institute, pp. 1-20. 1939.



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PRESERVATION OF BOVINE SPERMATOZOA IN YOLK-CITRATE DILUENT AND FIELD RESULTS FROM ITS USE

G. W. SALISBURY, H. K. FULLER, AND E. L. WILLETT

Department of Animal Husbandry, Cornell University, Ithaca, N. Y., and Seneca Cooperative Cattle Breeders' Association, Inc., Interlaken, N. Y.

INTRODUCTION

Scherstén (11) found appreciable quantities of citrates in the sexual gland secretions of man and animals and stated that a considerable portion of the buffer capacity of the semen was due to citrates. Others, including Slowtsoff (12), Huggins and Johnson (5), and Goldblatt (3), working with human beings, and McKenzie and co-workers (6), working with the boar, have analyzed semen, and their work shows that it contains considerable quantities of phosphates and carbonates. Willett (14) recently has determined the buffer coefficient curves for representative samples of the semen of the bull, the stallion, man, and the dog. The peaks of buffer capacity of bull semen coincide with the pH levels at which citrates, phosphates, and carbonates would be effective as buffers.

Scherstén (11) found that addition of sodium citrate to a Ringer-phosphate solution at the rate of 30 to 60 mg. per 100 cc. increased the longevity of spermatozoa suspended therein. Earlier Gray (4) had used sodium citrate to disperse spermatozoa agglutinated by metallic ions. However, Fleig (2) and Dubincik (1) have reported that citrate anion had a deleterious effect on sperm.

In light of these several investigations it was thought worth-while to develop a buffer mixture for the preservation of bull spermatozoa which would correspond closely to the buffers of semen to be used with egg yolk in place of the phosphate suggested by Phillips (8) and Phillips and Lardy (9), and which had given such splendid results in investigations by us (15).

LABORATORY STUDIES

An M/15 solution of sodium citrate and an M/15 solution of potassium di-hydrogen phosphate were added to egg yolk in such amounts as to make the resulting mixture contain about equal parts of egg yolk and buffer with

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the pH adjusted at 6.75. When first used it was noted that the new citrate buffer dispersed the fat globules and other materials in the yolk so that the resulting mixture was clear in appearance. When using the microscope to examine a sample of semen diluted with this mixture one could readily discern the individual sperm; on the other hand, when using the yolk-phosphate diluent it was necessary to further dilute the mixture with a clear diluter before the individual sperm could be distinguished.

Since this property was lost when the proportion of citrate was reduced by a small amount it was decided to use only enough potassium di-hydrogen phosphate as was compatible with satisfactory storage. Investigation showed that the phosphate buffer was not necessary for satisfactory results in the storage of semen when egg yolk was also used. Consequently, in the investigations herein reported the yolk-citrate diluent was composed of equal parts of fresh egg yolk and an M/15 solution of sodium citrate. Four or five parts of this diluent were added to one part of semen for the storage studies.

Comparisons were then undertaken to determine the relative value of the yolk-citrate diluent and the yolk-phosphate diluent in preserving the motility of spermatozoa under standard storage conditions. All the semen used in this investigation was collected from the dairy bulls in the Cornell University herd with the new-type artificial vagina (10). The semen was divided into equal portions immediately after collection. One-half was diluted at the rate of 1 part of semen to 4 or 5 parts of the yolk-citrate diluent. The other half was diluted at the same rate with yolk-phosphate diluent made after the directions of Phillips and Lardy (9).

The diluted samples were divided into 0.5 cc. portions and placed in small culture tubes. These tubes were then wrapped individually with paper and put into larger test tubes. The larger tubes were then placed in controlled-temperature water baths for cooling. Cooling was accomplished by changing the tubes from one bath to another at definite intervals. In these studies the semen was cooled from room temperature to the storage temperature of 5° C. at the rate of 5° C. per 10 minutes. After storage the samples were warmed at the rate of 10° C. per 10 minutes. Willett (14) has shown that, while one must carefully control the rate of cooling of semen for best results, the rate of warming the sample is apparently of little importance.

Each sample of semen was examined for motility at 37° C. in a microscope stage incubator immediately after collection and after 2, 4, 6, 8, and 10 days of storage. With the yolk-phosphate diluent it was necessary to further dilute the samples with SGC-2 diluter (Milovanov (7)) so that the spermatozoa might be readily seen. Studies of the buffer coefficient curves of this diluter showed that it possessed no appreciable buffer capacity and did not influence the pH of the stored semen plus yolk-diluent when added

to it. Estimations of motility were made on the basis of the proportion of actively motile spermatozoa and were expressed as per cent to the nearest unit of 10. Willett (14) has shown that this method of estimation of motility gives highly repeatable results.

RESULTS OF LABORATORY STUDIES

In table 1 is presented a summary of the studies comparing the motility of the spermatozoa during storage in the citrate and phosphate diluents. By the analysis of variance technique (13), no significant differences were

TABLE 1

Comparison of motility of spermatozoa during storage in yolk-phosphate and yolk-citrate diluents at 5° C. and without mineral oil

Time stored	Sample pairs	Motility means			Probabilities for differences between means
		Before storage	After storage		
			Phosphate	Citrate	
<i>days</i>	<i>No.</i>	<i>%</i>	<i>%</i>	<i>%</i>	
2	19	71	47	50	> .05
4	19	72	38	40	> .05
6	17	72	26	35	< .01
8	19	72	23	32	< .01
10	16	72	13	22	< .01

detected after 2 and 4 days of storage. After longer intervals of storage the differences were highly significant in favor of the citrate diluent. In addition, it was noted that, especially after 6 and 8 days storage, the spermatozoa were more active and many more showed progressive motility in the citrate than in the phosphate diluent.

In table 2 is presented a summary of the pH values of the semen-yolk-buffer mixtures before and after storage. It can be readily seen that there was practically no difference between the average pH values of the two mixtures either before or after storage for different intervals.

TABLE 2

Comparison of pH of semen during storage in yolk-phosphate and yolk-citrate diluents at 5° C. and without mineral oil

Time stored	Sample pairs	pH means			
		Before storage		After storage	
		Phosphate	Citrate	Phosphate	Citrate
<i>days</i>	<i>No.</i>	<i>pH</i>	<i>pH</i>	<i>pH</i>	<i>pH</i>
2	18	6.74	6.71	6.62	6.64
4	19	6.74	6.71	6.55	6.54
6	17	6.73	6.70	6.54	6.50
8	19	6.73	6.70	6.50	6.45
10	15	6.72	6.70	6.45	6.44

Although the average pH values of the mixtures before storage were about the same, the values for the semen in the citrate buffer were more variable than those for the phosphate buffers, for their coefficients of variation were 1.12 per cent and 0.73 per cent, respectively. This can be explained by the lower buffer capacity of the citrate diluent at these pH levels, with the result that the pH of the semen, which is quite variable, would have a greater influence on the pH of the citrate than on the phosphate diluent.

FIELD STUDIES AND RESULTS

Since the citrate buffer appeared to excel the phosphate buffer when used with egg yolk for the preservation of semen during extended storage, an experiment was designed to compare the fertility of spermatozoa stored for various intervals in the two diluents. This experiment was undertaken in cooperation with the Seneca (Seneca County, New York) Cooperative Cattle Breeders' Association, Inc., and all of the inseminations were carried out by H. K. Fuller.

The semen was collected and handled in a manner already described (15). Alternate ejaculates from each bull were preserved in the citrate and the phosphate diluents and then used for breeding after various intervals of storage. Semen from five bulls was used and the results for each bull are reported separately in table 3. Bulls number I and II are Holsteins, the others are Guernseys.

Save for the first twenty-four-hour period, the use of the citrate and phosphate diluents gave comparable results until the fifth day when the citrate gave slightly better results. However, this latter difference was not significant, nor were any of the other differences between the results obtained with the yolk-citrate and yolk-phosphate diluents significant when analysed with chi-square in a two by two table. Furthermore, when the values for all of the time intervals were totalled, the numbers of services required per conception with the semen preserved in the two diluents were practically identical. As far as these fertility studies go they tend to bear out the results of the storage studies which showed no significant differences until the sixth day and after. If the fertility studies had been extended over a longer period of storage the superiority of the citrate diluent which was indicated from the results of inseminations during the fifth day might have been established as real.

SUMMARY

1. An M/15 solution of sodium citrate mixed in equal amounts with fresh egg yolk produced a diluent which dispersed the fat globules and other material in the yolk so that when semen was diluted with it the individual spermatozoa could be readily seen upon microscopic examination.

2. The yolk-citrate diluent and the yolk-phosphate diluent were ap-

TABLE 3
Breeding results in the Seneca Cooperative Cattle Breeders' Association when comparing the yolk-phosphate and yolk-citrate diluents

Bull	Age of semen used (hours)								Services per conception						
	0-24		24-48		48-72		72-96			96-120		120-144		Totals	
	Serv.	Conc.	Serv.	Conc.	Serv.	Conc.	Serv.	Conc.		Serv.	Conc.	Serv.	Conc.	Serv.	Conc.
	Yolk-phosphate diluent														
I	10	6	8	6	14	7	6	4	11	7	2	1	51	31	1.65
II	1	1	9	6	4	3	6	4	6	3	9	4	35	21	1.67
III	5	5	9	5	10	8	8	7	4	2	36	27	1.33
IV	6	4	10	7	7	4	5	4	2	2	30	21	1.43
V	2	1	7	6	10	6	11	8	9	5	2	0	41	26	1.58
Totals	24	17	43	30	45	28	36	27	32	19	13	5	193	126
Serv. per conc.	1.41		1.43		1.61		1.33		1.68		2.60		1.53	
Yolk-citrate diluent															
I	16	13	13	14	8	16	10	1	0	47	31	1.52
II	4	5	3	3	13	8	16	11	9	6	5	3	52	35	1.49
III	5	11	8	4	4	1	2	2	1	0	23	13	1.77
IV	2	0	15	10	5	4	4	4	4	1	30	19	1.58
V	1	1	8	7	8	5	5	3	4	4	26	20	1.30
Totals	55	41	41	44	26	43	30	19	11	5	3	178	118
Serv. per conc.	1.71	1.34			1.69		1.43		1.73		1.67		1.51	

parently equal as preservatives of the motility of spermatozoa which were stored in them under standard conditions for two and four days.

3. The yolk-citrate diluent was superior to the yolk-phosphate diluent for the preservation of motility when semen was stored six days or more.

4. In actual insemination tests with semen stored up to 5 days no significant difference in fertility was found between the semen stored in the yolk-citrate and the yolk-phosphate diluents.

5. The results of the fertility studies tend to bear out the results of laboratory storage studies on the maintenance of motility under standard conditions.

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THE BACTERIOLOGY OF BULL SEMEN

I. C. GUNSAIUS, G. W. SALISBURY, AND E. L. WILLETT

*Laboratory of Bacteriology and Department of Animal Husbandry,
Cornell University, Ithaca, New York*

Practically all workers in the rapidly expanding field of artificial insemination stress the necessity of bacteriological control. It therefore seems desirable to know definitely what measure of bacteriological control is necessary or desirable in the collection, handling, and storage of semen. In addition, information on the types and numbers of bacteria present under varying conditions of collection and storage and their possible relation to infection in the female genital tract would be useful. Attention was earlier called to these facts by Salisbury, Willett and Gunsalus (14).

Most of the available information on the bacteria of the reproductive tract or semen of bulls deals either with the incidence and spread of disease, such as Bang's disease, or with possible causes of sterility of bulls with poor breeding records. Little attention has been given to the subject in relation to the preservation of semen and to artificial insemination.

Various methods have been used to obtain semen for bacteriological study. Webster (17), Gilman (4), and Williams and Kingsbury (19) recovered semen from the vaginas of cows immediately after normal service. The vaginas and sheaths having previously been douched and disinfected as suggested by Williams. Gilman, and Williams and Kingsbury reported finding micrococci, hemolytic and non-hemolytic streptococci, coliform organisms and *Brucella abortus*. They concluded that the genital tract and semen of normal bulls contained few, if any, bacteria, but that sterile bulls or those of diminished fertility usually contained large numbers. Webster reported that on culture normal semen yielded diphtheroids and micrococci, while bulls from herds in which enzootic sterility was present produced semen which contained, in addition, many alpha hemolytic streptococci. As suggested by Gilman, bacteriological studies on semen obtained by this technique are not entirely satisfactory due to possible contamination from the vagina.

Gilman (3) also studied the genital tracts of bulls shortly after slaughter and reported the presence of *Pseudomonas pyocyaneus* and unidentified rods in addition to the micrococci, streptococci, and coliform organisms. His findings also indicated that the genital tracts of normal healthy bulls were either free from bacteria or contained very low numbers, whereas impotent bulls harbored large numbers of organisms which were undoubtedly ejaculated with the semen. Gilman considered infection to be the greatest single

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cause of functional and anatomical changes resulting in varying degrees of impotency.

Hatziolos (7) collected semen by use of the artificial vagina and attempted to obtain the semen as free from bacterial contamination as possible. The bacteria, which he found in every ejaculate, consisted mainly of coliform organisms, the proteus, and pseudomonas groups, cocci and spore forming rods.

That these types of bacteria can be responsible for metritis, cervicitis, sterility, or abortion in cows has been indicated by the observations of Lucet (9), Rosenow and Davis (12), Beaver, Boyd, and Fitch (1), Udall, Cushing and Fincher (16), and others.

Semen is a rather good medium for the growth of bacteria. Probably the first to report such an observation was Spallanzani (15) who in 1785 observed that the sperm from the terrestrial fetid toad soon became putrid. He attributed the diminishment of fertility of the sperm during storage to this putrefaction. Roemmele (11) noticed bacteria in semen stored at room temperature and at 9° C., and attributed the death of the sperm to the accumulation of the metabolic products of the bacteria. Hammond (6) observed bacterial growth in samples stored at 10° C., but found no greater incidence of infection in female rabbits inseminated with semen in which large numbers of bacteria had developed than with samples where fewer organism had grown due to a lower storage temperature. Krzyzskowsky and Pawlow (8) also mentioned that bacteria grew rapidly in semen, especially a small motile bacillus which formed a blue-green pigment (probably *Pseudomonas pyocyaneus*), though no definite data could be found to indicate whether bacteria have a direct detrimental effect on spermatozoa. Krzyzskowsky and Pawlow (8) thought that the products of metabolism of bacteria influenced the spermatozoon life. Gunn (5) on the other hand could detect no influence of bacteria on the survival of spermatozoa stored at low temperatures, (4° C.).

METHODS

The semen used in these studies was collected with the new-type artificial vagina (Salisbury and Willett (13)). The rubber liner of this device was sterilized by flushing with alcohol and drying before use. All glassware with which the semen came in contact was sterilized with dry heat. The mineral oil, petrolatum used as a lubricant, Milovanov's (10) SGC-2 diluent and phosphate buffers were sterilized by autoclaving.

The numbers of bacteria were determined by plate counts on blood agar containing 2 per cent sterile defibrinated horse blood. All plates were incubated 4 days at 37° C. This incubation period was selected in order to facilitate counting when slow growing diphtheroid organisms were present. The relative numbers of coliform bacteria were determined by serial dilutions in Durham tubes of glucose broth.

EXPERIMENTAL

Numbers and Predominating Types of Bacteria. The semen used in these studies was obtained from bulls in the University dairy herd and from bulls in the Central New York Artificial Breeders' Cooperative at Syracuse. The bulls at the University were used in the breeding program of the herd more or less frequently and bred the cows naturally. Since these bulls had access to exercise lots which often were muddy, their underlines and sheaths were frequently dirty. Unless the bulls at Syracuse had been brought into the herd only a short time before the samples were obtained they had not bred cows naturally for considerable periods of time, unless they had accidentally bred a cow in the breeding rack while the artificial vagina was being used. In addition, they were confined to stalls during the major portion of the day and were out-of-doors only while being exercised on a mechanical exerciser. As a result these bulls were clean about the underline and sheath.

TABLE 1
Number and kinds of bacteria found in fresh bull semen

Bull	Number ejaculates	Bacteria per cc. of semen (in thousands)		Predominating kinds
		Average	Range	
1	1	650	<i>Pseudomonas</i> , <i>E. coli</i>
2	2	7	1- 12	Diphtheroids
4	1	20
6	1	15	Diphtheroids
7	2	2	1- 2	" , <i>Staphylococci</i>
8	1	750	<i>Pseudomonas</i> , <i>E. coli</i>
9	1	30	Diphtheroids
21	1	90	" , <i>Bacilli</i>
22	2	6	1- 12	"
23	1	1
24	1	4,300	Diphtheroids, <i>Pseudomonas</i> , <i>E. coli</i>
26	1	50	" , <i>Staphylococci</i> , <i>E. coli</i>
D	5	1,270	13- 4,900	"
E (1939)	6	4,600	290-22,000	" , (some hemolytic)
E (1940)	6	230	10- 750	" , <i>Pseudomonas</i>
F	2	120	5- 230
H	4	6,500	10-20,000	Diphtheroids
I	2	260	130- 390
J	1	1	<i>Pseudomonas</i>
K	2	720	690- 750

The data in table 1 indicate the numbers and predominating kinds of bacteria found in fresh ejaculates when no precautions were taken in cleaning the underline or sheath of the bulls prior to collection. Of the 12 bulls in the artificial breeding herd (represented by numbers in table 1), two-thirds gave plate counts of fifty thousand bacteria per cc. or less and only 3 gave plate counts of over one hundred thousand. One of these (number 24), which gave semen with over four million bacteria per cc., had not been

used previously for over three weeks, having just been added to the herd and not as yet put into use. All the other bulls had been used an average of once to twice per week before the samples were taken for examination. The other two samples with extremely high counts (numbers 1 and 8) contained large numbers of coliform and pseudomonas organisms as did number 24. All three of these bulls have since been removed from use in the artificial breeding circuits. None of the other samples contained as many as 100 coliform organisms per cc. The average of the logarithms of the plate count of these 15 samples was 22 thousand bacteria per cc. Of 28 semen samples from 7 bulls in the University herd (represented by letters in table 1), the majority contained from 100 thousand to several million bacteria per cc. Only four samples contained as few as ten thousand bacteria per cc. The average of the logarithms of the bacteria in the individual samples was 225 thousand per cc. whereas the arithmetic average of the counts was 2.3 millions. In comparison with the bulls in the artificial breeding circuit, these bulls produced semen containing 10 to 50 times as many bacteria. Whether this is due to frequency and kind of service (natural and artificial vagina) or other factors is not elucidated in this study.

The types of bacteria reported in these samples are only those which occurred in large numbers and not all types present. All the types reported by other workers as frequently found in semen have been found with the exception of streptococci. The organisms occurring most regularly in large numbers were diphtheroids. Hemolytic diphtheroid organisms were isolated from one bull (E) when one series of samples was taken, but they were not found a year later when a second series of samples were studied.

The number of bacteria present in these samples was higher than would have been expected from the reports of a number of workers who considered that semen from normal healthy bulls is almost free from bacteria. *Pseudomonas pyocyaneus* was isolated from five of the nineteen bulls studied. In this connection it should be noted that three of the four bulls dropped from breeding operation of the Central New York Artificial Breeders' Cooperative within the last six months were among the five in the semen of which pseudomonas organisms were present in large numbers. Of the other two bulls, one has since become sterile and the other has a record of 12.1 per cent conception from 91 inseminations by artificial breeding. Gilman (3) considered these organisms as among those of importance in genital infections.

Effect of Cleaning the Bull. In order to determine whether it would be possible to reduce the number of bacteria in semen collected with the artificial vagina, two bulls were cleaned, to the extent of thoroughly washing the underline with soap and water, clipping the hair from about the prepuce, and flushing the sheath with sterile distilled water. When bull (D) was treated in the above manner on alternate weeks, the first sample being col-

lected without cleaning the bull, the counts were as follows: 490, 3, 32, 3 in thousands. The reduction in count by the first cleaning was over a hundred fold and in the second case 10 fold, the count not having reached as high a value one week after cleaning as before any treatment. A second bull (I) which gave semen with a lower count without cleaning than D, did not show as great response to cleaning, the counts on consecutive weeks being 13, 1, 39, 6 in thousands. The bull was cleaned before the second and fourth collection. Here the reduction was about 10 fold, the counts being reduced to about the same number as those obtained when bull D was cleaned.

Preparation of Yolk-phosphate Diluent. It was found during the early course of the experiment that aseptic methods must be practiced in order to produce a yolk-phosphate diluent without undue contamination. To produce such a diluent, fresh eggs were obtained from a pullorum-free flock and immersed in normal NaOH or 70 per cent alcohol to sterilize the shell (Bryant and Sharp (2)). The eggs were then broken and the yolk removed, using sterile glassware, and mixed with sterile buffer. By using these precautions yolk-phosphate diluents with very low bacterial counts, could be consistently produced. Thus, of 21 samples of diluent so prepared, 17 had counts between 0 and 15 bacteria per cc., 2 of 150 and 200, and 2 had counts of 1,000,000 and 7,000,000. The last two samples mentioned may have been contaminated by the equipment or the buffer, for immediately after they occurred all the material was sterilized with the result that 12 consecutive samples were prepared during the following month with no count over 200. Four samples prepared with unsterilized buffer had counts ranging from 600,000 to 4,000,000, and two samples prepared with yolk from store eggs without sterilizing the shell or the buffer had counts of 24,000,000 and 166,000,000. As will be shown below, semen-yolk-phosphate mixtures containing such large numbers before storage often had counts in the billions after storage for several days. The yolk-phosphate diluent may be the important source of large numbers of bacteria in stored semen samples if care in preparation is not exercised.

Growth of Bacteria in Semen During Storage. The following studies were performed to investigate the growth of bacteria in undiluted or diluted semen during storage.

In one experiment 4 ejaculates were each divided into 3 parts and stored undiluted, in SGC-2 diluent and in yolk-phosphate diluent at 5° C. and 10° C. Samples from each treatment were examined after 4 and 8 days of storage. The data are summarized in table 2. It can be seen that the yolk-phosphate diluent was an excellent medium for bacterial growth as evidenced by the high count after storage for 8 days at 10° C. The bacteria grew least rapidly in the undiluted semen. As would be expected, there was considerably more bacterial growth at 10° C. than at 5° C. In fact, at the lower tem-

TABLE 2

Effect of storage temperature and diluent on bacterial growth (averages from 4 ejaculates)

Treatment	Bacteria per cc. in thousands after storage				
	5° C.			10° C.	
	0 days	4 days	8 days	4 days	8 days
Undiluted	190	420	400	430	740
Yolk-phosphate	200	250	520	600	12,000
SGC-2	100	130	80	310	2,600

perature there was not enough bacterial growth to effect changes in the medium. Therefore 5° C. or lower is preferable as a storage temperature in order to hold the bacterial growth to a minimum, especially since it has been shown by Willett (18) that storage at 5° C. preserves the viability of the sperm longer than higher storage temperatures.

During the course of Willett's study on the preservation of semen, other observations were made in regard to the growth of bacteria in semen-yolk-phosphate mixtures stored under different conditions. A comparison was made to determine if covering the diluted semen samples with a layer of mineral oil would reduce the growth of bacteria when they were stored for 4 and 8 days as had been indicated by certain preliminary observations. The data, from counts in 9 ejaculates, summarized in table 3 indicated that there was not enough difference between the two treatments to be of practical importance. Also, during the course of these bacteriological investigations several ejaculates were stored in the yolk-phosphate diluent in which the buffer was not sterilized. It was noted that the bacterial counts of such samples during storage reached numbers far exceeding those samples made with sterilized buffer. A summary of these counts is presented in table 3 for comparison with the counts of other uncontaminated samples. Since some workers in the field of artificial insemination recommended temperatures lower than 5° C. for semen storage, samples for 7 ejaculates were stored at both 5° and 1° C., and the bacterial counts compared after 4 and 10 days of storage. It can be seen (table 3) that there was no appreciable difference in numbers of bacteria after storage at the two temperatures.

During these studies of bacterial growth during storage, observations of spermatozoon motility and pH measurements were also made concurrently. The data show no definite relationship between the numbers of bacteria present and the motility of the spermatozoa or pH of the semen during storage. However, because we were largely successful in bacteriological control there were only a few samples in which enough bacteria developed to cause such changes.

The bacteria from bedding, manure, soil, unsterilized glassware, etc., would include *E. coli*, bacilli, and staphylococci. These are the types which

TABLE 3
Bacterial growth during storage in semen yolk-phosphate diluent

Sample pairs	Treatments	Bacteria per cc. (in thousands)					
		Before storage		After storage			
		Average	Range	4 days		8 days	
				Average	Range	Average	Range
No. 9	Mineral oil, 5° C.	582	1-2,550	293	2- 2,100	426	6- 1,300
	No mineral oil, 5° C.	"	"	452	3- 3,550	533	4- 2,250
6	Diluent contaminated, 5° C.	2,490	170-5,500	165,000	100- 580,000	2,910,000	100- 7,150,000
	"	"	"	464,000	100-2,040,000	12,000,000	175,000-28,500,000
7	5° C.	367	1-1,350	55	3- 105	341	4- 1,000
	1° C.	"	"	47	2- 145	352	2- 1,150

can be eliminated or materially reduced in number by the methods outlined above, and are the types which grow readily at storage temperatures. Therefore, if contamination by these types can be prevented, little bacterial growth will take place in the semen or semen-yolk-phosphate mixture during storage. On the other hand, it appears that the diphtheroids are always present in semen in variable numbers no matter what precautions are taken. However, this type does not grow at the usual low storage temperatures, and therefore, does not become numerous during long storage. In a number of samples pseudomonas organisms were present in large numbers. Since they grow at low temperatures, one would not be able to eliminate growth of these bacteria by using such temperatures.

Germicidal Action of Semen. In the storage experiments above reported it was observed that the number of bacteria decreased during storage in some cases. The question arose as to whether semen had a bactericidal action. Several experiments were performed to see if a bacteriacidal effect could be demonstrated at storage temperatures.

Semen from each ejaculate was divided into two parts. One part of the semen was stored untreated, while the other was inoculated with a culture of *Escherichia coli*. SGC-2 diluent inoculated with *E. coli* served as a control. The samples were stored between 5° and 7° C. without mineral oil in 25 cc. test tubes plugged with cotton. At the start of the storage period the counts were made every one-half or one hour, but the intervals were lengthened with the progress of the storage period. In three studies thus made at these low temperatures, there was no evidence of any germicidal action.

During these experiments frequent observations of spermatozoon motility were also made with rather startling results. It was found in all three experiments that the motility of the spermatozoa in the inoculated samples was markedly superior to those in the untreated semen when diluted and examined after storage. In one experiment a sample of semen was inoculated with coli killed by heating to compare with the other treatments. The spermatozoa in the sample showed no greater motility than the untreated semen.

SUMMARY

1. The bacterial count on 43 ejaculates collected from 19 bulls by means of an artificial vagina ranged from 1,000 to 22,000,000 per cc.
2. It was found that by douching the sheath and washing the underline, if the bull was dirty, the number of bacteria in semen could be markedly reduced.
3. Almost sterile yolk-phosphate diluent was consistently produced when fresh eggs from healthy hens were used and when aseptic methods were employed in the preparation of the diluent. Under other conditions the

diluent may be responsible for the addition of large numbers of bacteria to semen samples.

4. Bacterial growth during storage was held at a minimum by storing at 5° C. or lower.

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PSEUDOMONAS PUTREFACIENS IN DAIRY PLANT EQUIPMENT*

H. F. LONG AND B. W. HAMMER

Dept. Dairy Industry, Iowa State College, Ames

In outbreaks of putrid butter the sources of the causative organisms undoubtedly vary. Some outbreaks have been traced to water used to wash the butter or equipment (1, 3), while in other instances water has not been involved. *Pseudomonas putrefaciens* commonly is responsible for the putrid defect in salted butter (1, 2), at least in certain areas. It is sensitive to heat, being killed in 2 minutes at 61.7° C. (4), so that it probably never survives pasteurization. A source other than water which could contribute the organism to pasteurized cream or to butter is dairy plant equipment. Attempts to isolate *Ps. putrefaciens* from such equipment are reported herein.

METHODS

Frequently, bacteriological examinations of dairy plant equipment have involved rinsing with sterile water and then culturing the water. In the case of churns this is unsatisfactory because many organisms are between staves, at junctions of ends and staves, around bolts, etc., from which they are dislodged only when there is considerable strain on the churn, for example, when the butter breaks or when it is worked. In these locations the organisms are protected during the attempts to sterilize the churns, and their multiplication continues to supply organisms for the contamination of one lot of butter after another. Much the same situation probably exists with certain other equipment, especially leaky vats.

In the isolation studies reported herein such materials as curd and wood from various points in the equipment were smeared directly, and also after enrichment in litmus milk at 3° C. for varying periods, on a special medium (4) consisting of: gelatin 4 per cent, proteose peptone 2 per cent, dipotassium phosphate 0.1 per cent, ferric ammonium citrate 0.05 per cent, agar 1.5 per cent and water to make 100.0 per cent. On this medium *Ps. putrefaciens* grows relatively well at 21° C. and gives colonies with a rather characteristic appearance. Colonies suggesting *Ps. putrefaciens* were purified and enough of the characters studied to determine whether the organisms belonged to this species.

RESULTS

In some instances *Ps. putrefaciens* was obtained from dairy plant equipment and in other instances isolation attempts failed. Examples of both successful and unsuccessful attempts are as follows:

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Example 1. In a plant making butter that kept satisfactorily in normal marketing channels, an occasional churning became putrid in 5 to 7 days in keeping quality tests at 21° C. Pasteurization of the cream appeared to be efficient, and the wash water was chlorinated. Materials were collected from various parts of two of the several churns in the plant by loosening the shelves, removing bolts, etc.

With churn 1, *Ps. putrefaciens* was recovered by direct smears from between the end of a shelf and the iron cover plate and from wood near a bolt; it was recovered by enrichment from three of four points near bolts; and it was not recovered from five points, including the buttermilk drain, around the sight glass, under a bolt head and on the brace (two points) under a shelf. With churn 2, the organism was recovered directly from the end of the shelf and from under the shelf but not from two other points near the shelf; enrichment did not detect additional positive samples.

Microscopic examinations of the original material indicated that micrococci, gram-positive rods, gram-negative rods, yeasts and molds were present, often in large numbers; these general types of organisms also were evident on the plates.

Example 2. Some months after the collection of the samples referred to in example 1, the spoilage of butter in the keeping quality tests no longer occurred in the plant. Because of its general condition it was necessary to replace an end in one of the churns and during the operation six samples were obtained from around bolts, under the sight glass and at the junction of shelves and ends. None of the samples yielded *Ps. putrefaciens*, either directly or by enrichment. All the plates were heavily overgrown with various microorganisms.

Example 3. A coil vat in a milk plant was discarded because of leaks which permitted liquid to soak into the insulation. Within 24 hours of the last use of the vat, holes were cut through the outer wall and samples of the insulation collected. Near the leaks the insulation was soaked with water and had a very objectionable putrid odor, while at some distance from the leaks it was moist and the odor was somewhat putrid.

Ps. putrefaciens was isolated by direct smears from four of the seven samples but was not obtained by enrichment from any of them. Three of the four positive samples were from portions of the insulation that were very wet, while one was from a portion that was only moist.

After the insulation had dried rather completely over a period of 5 weeks, eight additional samples were collected. Cultures of these, both without and with enrichment, failed to show *Ps. putrefaciens*, the plates being largely overgrown with aerobic spore-forming bacteria.

Example 4. A churn barrel was taken out of a plant because the general condition, particularly of the roll, made repairs inadvisable. The plant had not experienced the putrid defect; however, the butter was consumed

quickly and keeping quality tests were not being run. Since the barrel was to be used for holding water it could not be dismantled and only the roll and one shelf were removed. Material was collected from one end of the roll, from one end of the shelf, from around the shelf support and from around bolt heads.

Ps. putrefaciens was not detected on plates smeared with the different samples, either directly or after enrichment. However, the plates were heavily overgrown with various microorganisms, including micrococci, spore-forming bacteria, gram-negative rods, yeasts and molds.

Example 5. A plant experiencing a serious outbreak of putrid butter quickly brought the difficulty under rather complete control as far as spoilage in commercial channels was concerned. However, many churnings continued to show spoilage after 5 or 6 days in keeping quality tests at 21° C. Various procedures, such as chlorination of the water, unusually high pasteurization of the cream, etc., failed to prevent the spoilage in the keeping quality tests. The barrel of the older of the two churns in the plant was replaced and about 15 inches of one end of the barrel was cut off and sent to the laboratory. Over a period of several days, 67 samples were collected from various parts of the end which was completely broken up in the process.

Ps. putrefaciens was recovered directly from 13 of the samples; 8 of these were from points at the junction of staves and the end of the churn, 2 from between boards in the end of the churn, 2 from under the sight glass and 1 from wood near a bolt. After enrichment the organism was obtained from 3 additional samples, 2 being from points at the junction of staves and the end of the churn and 1 from between boards in the end of the churn. In some instances *Ps. putrefaciens* made up a rather large percentage of the colonies on the plates smeared directly with a sample. The flora of many of the plates also included micrococci, spore-forming bacteria, gram-negative rods and occasionally some yeasts and molds.

DISCUSSION

The isolation of *Ps. putrefaciens* from various points in churns and from the insulation of a leaky vat indicates that equipment may be a source of the organism in dairy products. Evidently *Ps. putrefaciens* survives the cleaning and heating of equipment due to protection afforded in crevices, cracks and joints. The churns from which *Ps. putrefaciens* was isolated were reported to have received thorough and regular cleaning.

The fact that plates smeared with material from churns not yielding *Ps. putrefaciens* often were heavily seeded with other species, many of which were not heat resistant, is additional evidence that conditions in certain churns may be favorable for *Ps. putrefaciens*. It also suggests that equipment may yield other organisms capable of causing defects in dairy products.

The flora of certain plates smeared with material from churns definitely

resembled the flora of plates prepared from putrid butter. Such plates, regardless of whether or not *Ps. putrefaciens* is present, usually contain micrococci, spore-forming bacteria, gram-negative rods and often yeasts and molds. This suggests that with defective and highly contaminated butter much of the contamination may originate in the churn.

SUMMARY

Ps. putrefaciens was isolated from churns and from the insulation of a leaky vat. At certain points the organism was present in considerable numbers. In addition to *Ps. putrefaciens*, micrococci, spore-forming bacteria, gram-negative rods and often yeasts and molds were present.

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OXIDIZED FLAVOR IN MILK. IX. THE EFFECT OF THE
QUALITY OF HAY AND EARLY STAGE OF LACTATION
ON THE CAROTENE CONTENT OF BUTTERFAT AND
ON THE ASCORBIC ACID CONTENT OF THE MILK
AND THEIR RELATIONSHIP TO THE DE-
VELOPMENT OF METAL-INDUCED
OXIDIZED FLAVOR*

W. CARSON BROWN,¹ A. H. VANLANDINGHAM² AND CHAS. E. WEAKLEY, JR.²
West Virginia Agricultural Experiment Station, Morgantown

One of the earliest workers to report a seasonal variation in oxidized flavor was Mattick (11) who in 1927 reported that oiliness in milk appeared in autumn, winter and spring, but never in the summer. Kende (10) recognized this fact and as a result of his work concluded that green feed contained some substance or substances which when fed to the cow protected the milk against the off-flavor. Since that time numerous workers have observed the difference in the susceptibility of winter and summer milk to oxidized flavor. Stebnitz and Sommer (15) found that when cows received grass as part of their ration, the butterfat became less saturated and more susceptible to oxidation. However, it appeared that protective substances in the milk prevented the development of oxidized flavor. Likewise, it is generally agreed that green feeds yield a more stable flavored milk as compared to milk produced on dry feed.

The nature of these inhibiting substances carried in green feed has been the object of extensive investigations. Anderson and co-workers (1, 2) were some of the earliest investigators to show the effect of carotene on the milk flavor. Their work showed that carrots or machine-cured alfalfa fed to cows would eliminate oxidized flavor in their milk. This they correlated with the carotene content of the feed. Anderson, Hardenbergh and Wilson (2) obtained far more effective results in the elimination of oxidized flavor by feeding 8 pounds of carrots in the daily ration than by feeding a cod-liver oil concentrate containing 500,000 U.S.P. units of vitamin A. Whitnah and co-workers (17, 18) and Beck, Whitnah, and Martin (4) report that a carotene supplement quickly corrected the tendency for oxidized flavor to develop spontaneously. In addition they found oxidized flavor more prevalent in milks that were below the breed average in fat color intensity. There were, however, samples low in color which did not develop oxidized flavor. Likewise, it was noted that the color of the fat was not increased for some time

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¹ Department of Dairy Husbandry. ² Department of Agricultural Chemistry.

after the flavor was improved. These workers (18) report that low-carotene intake levels were not the only factor determining the tendency for milk to develop oxidized flavor as shown by the fact that the five lowest carotene intakes per kilogram of body weight among cows producing milk that kept a good flavor were below the three highest intakes among these cows producing milks which developed oxidized flavor.

Brown, Vanlandingham, and Weakley (6) found that a carotene supplement added to the ration rendered the milk less susceptible to metal-induced oxidized flavor. Likewise, the supplementing of a low-carotene ration with ascorbic acid produced similar results. Garrett, Tucker, and Button (9) found a positive correlation between color and flavor and between ascorbic acid and flavor. They concluded that high carotene and high ascorbic acid are coincidental to and help to preserve good flavor in milk. Garrett, Arnold, and Hartman (7) reported that alfalfa silage is almost equal to spring pasture in putting yellow color into milk and is equal to or better than pasture in producing milk of fine flavor and high resistance toward the development of oxidized flavor.

Brown, Thurston, and Dustman (5) found that feeding of one quart per animal per day of either tomato or lemon juice to cows on dry feed reduced the susceptibility of the milk to oxidized flavor. They attributed this effect to ascorbic acid in the feed and observed a similar tendency when pure crystalline ascorbic acid was fed at the rate of $\frac{1}{2}$ gram daily. Nelson and Dahle (12) added orange and tomato juice directly to the milk and observed a slightly greater protective effect than could be accounted for on the basis of their ascorbic acid values.

Garrett, Tucker, and Button (9) obtained information based on the average flavor scores which showed that for a decrease in ascorbic acid there was also a corresponding decrease in flavor score. The apparent critical point of the relationship of ascorbic acid to good flavor was found to lie between 15 and 18 mg. of ascorbic acid per liter of milk. Garrett, Arnold, and Hartman (7) reported that feeding grass silage had a greater stabilizing effect on ascorbic acid in milk than corn silage or beet pulp. This stabilizing effect tended toward milk of better flavor.

It has been shown that feeding carrots or machine-cured alfalfa hay will render milk non-susceptible to oxidized flavor and that both carotene and ascorbic acid in the feed play a role in the susceptibility of the milk to the flavor. Since the carotene in the butterfat is affected by the carotene in the feed, and since good quality hay is known to contain relatively large amounts of carotene, it appears that there should be a relationship between the quality of hay and the susceptibility of the milk to oxidized flavor. Since many producers who have difficulty with oxidized flavor purchase hay, it seemed desirable to know if the feeding of very high quality alfalfa hay would render the milk non-susceptible to metal-induced oxidized flavor. Accordingly, the following experiment was planned and conducted.

EXPERIMENTAL

For use in this experiment, eight Jersey cows, whose milks were susceptible to oxidized flavor when contaminated with copper, were selected and placed on a ration low in carotene. The feed consisted of a grain mixture of 100 lb. ground oats, 100 lb. wheat bran, 15 lb. cottonseed meal, 3 lb. salt, and 2 lb. of steamed bone meal, with different quality alfalfa hay and beet pulp as the roughage.

Three quarts of milk were collected from each cow at the morning milking on the first three consecutive days each week, and carotene, ascorbic acid, and flavor determinations were made. The ascorbic acid was determined on the individual samples of raw milk, as soon as possible after milking, by titrating as suggested by Sharp (16). The remaining milk was pasteurized in bottles. Following pasteurization and cooling, four one-half pint samples were prepared containing none, 0.5, 1.0, and 1.5 parts per million, respectively, of copper from a copper sulphate solution. These samples were then stored in ice water for three days, after which they were scored for flavor by at least three judges familiar with oxidized flavor. The remainder of the milk was prepared for carotene analysis by gravity separation of the cream followed by churning. Before churning, the cream from each of the three days was composited so as to make one churning and one analysis for carotene per cow per week. The butter thus obtained was melted and centrifugalized in Hart's casein tubes in an electrically heated centrifuge for 15 minutes, after which the clear, liquid butter oil was decanted into a clean, dry jar. The carotene analyses were made according to the method of Baumann and Steenbok (3) modified by Rogers and associates (14). After a 5½-week preliminary period on the regular herd ration the animals were changed to the experimental depletion ration for a period of four weeks. This ration consisted of the grain mixture already described, and eight pounds of brown alfalfa hay which still retained its leaves, together with 12 pounds of dry beet pulp.

At the end of the depletion period the animals were divided into 2 groups which were approximately equal in intensity of oxidized flavor. One group was continued on the brown hay roughage while the other group was changed to bright green, leafy, alfalfa hay. The hay given both groups was increased from 8 to 12 pounds per animal per day. Special care was taken to select hay of equal leafiness in both types of hay. The brown hay had an average carotene content of 5.8 mg. of carotene per kilogram whereas the green hay had an average carotene content of 43.0 mg. of carotene per kilogram. After two weeks on this ration the cows that were receiving the bright green hay were given hay *ad lib.* for three weeks and then were supplemented with 2 pounds of alfalfa leaf meal (carotene content 4.9 mg. of carotene per kilogram) daily. The purpose of this supplement was to increase the amount of alfalfa and carotene in the ration. Unfortunately, the amount of caro-

tene in the leaf meal was lower than expected and therefore the carotene supplement was not as great as was intended. However, there was a marked reduction in the intensity of the oxidized flavor of the milk.

After the three-week period in which the cows received leaf meal supplement, there was a readjustment period of three weeks followed by pasture for one week, after which time the experiment was discontinued. The cows

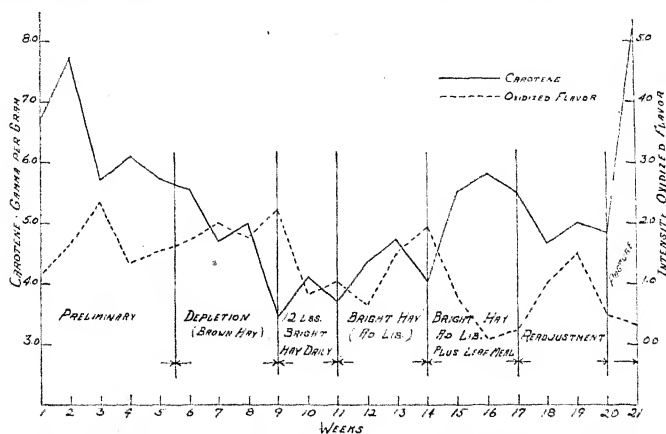


FIG. 1. The relationship between carotene and metal-induced oxidized flavor in the milk produced by cows on bright, green, alfalfa hay.

that received the brown hay were continued on the same basis except that they received no alfalfa leaf meal supplement. These animals were on pasture one week before the experiment was discontinued. The results of this experiment are shown graphically in figures 1 and 2. Figure 1 gives the average of the results obtained with the group on bright green hay while figure 2 gives the average results obtained on brown hay during the same periods.

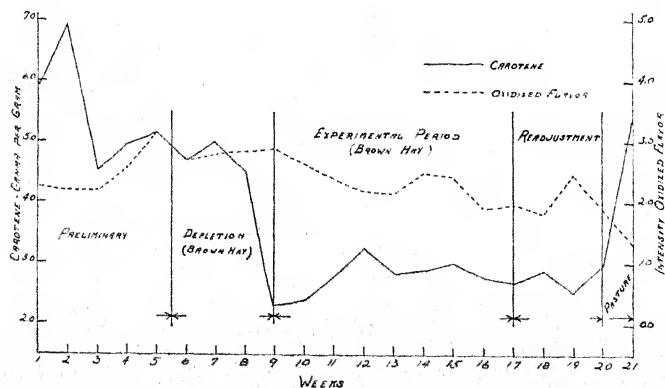


FIG. 2. The relationship between carotene and metal-induced oxidized flavor in milk produced by cows on a low-carotene ration receiving brown hay.

The results shown in figure 1 indicate that there may be a relationship between the carotene in the butterfat and the intensity of oxidized flavor developed, but this relationship is not very clear. During the first nine weeks there was a great reduction in the carotene of the butterfat, but there was no significant increase in the susceptibility of the milk to oxidized flavor. When the ration was supplemented with alfalfa leaf meal even though relatively low in carotene *per se*, there was an increase in the carotene of the butterfat and a corresponding decrease in the intensity of the oxidized flavor until it was almost eliminated. It may be observed, however, that the carotene in the butterfat, during the period when the ration was supplemented with alfalfa meal and practically devoid of oxidized flavor, was not as high as at the beginning of the experiment, when the oxidized flavor was relatively strong.

Figure 2 shows the relationship between the carotene content of the fat and the intensity of oxidized flavor of milk produced on brown hay. Here there seems to be an absolute lack of relationship, since the carotene content of the milk was greatly reduced and maintained at a low level for some time. It might be expected that the intensity of the oxidized flavor would be increased and that possibly some spontaneous oxidized flavor development might occur. However, examination of the data reveals that, contrary to the expected results, we find if anything a slight reduction in the intensity of the oxidized flavor developed.

There was no apparent relationship between the ascorbic acid content of the milk and the susceptibility of the milk as influenced by the quality of hay.

STAGE OF LACTATION

While the experiment on the quality of hay was in progress it was noticed that certain animals which had recently calved were producing milk relatively high in carotene but nevertheless with a strong oxidized flavor. It was decided to study the effect of early stage of lactation on the development of this flavor. Accordingly, samples were taken from nineteen cows which had just freshened on the herd ration. Carotene, ascorbic acid, and metal-induced oxidized flavor development in their milks were studied. All determinations were made in the same manner as in the previous experiment. The results obtained from the 19 cows studied are shown in figure 3. Examination of these data reveals that there was a marked decrease in the carotene of the butterfat for the first three weeks of lactation, after which it remained fairly constant for the next eight weeks. The intensity of the oxidized flavor developed followed a very similar curve and almost paralleled the carotene curve. In contrast to the results of Rasmussen *et al.* (13), it was found that the ascorbic acid content at the beginning of lactation was unusually low and increased gradually until it reached a maximum level at about eight weeks following parturition. These results verify the work of Whitnah and Rid-

dell (19), who found an average increase of 10 per cent in vitamin C concentration from the first to the second month of lactation. In this experiment there appeared to be an inverse relationship between the amount of ascorbic acid in the milk and the intensity of the metal-induced oxidized flavor.

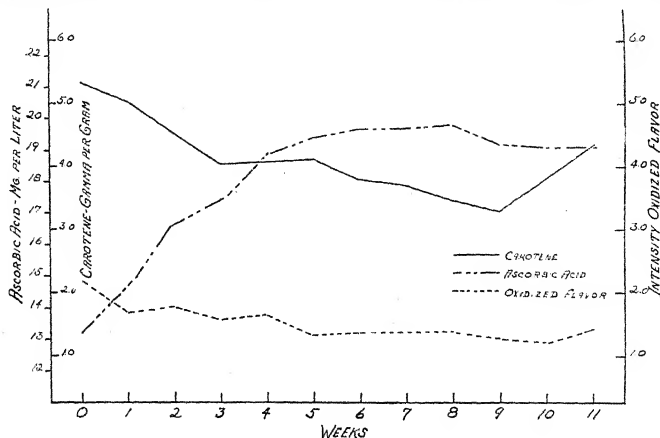


FIG. 3. The relationship between the carotene content of the butterfat, the ascorbic acid content of the milk, and the intensity of metal-induced oxidized flavor, as shown during the early stages of lactation.

Since there was no apparent relationship between the ascorbic acid content of the milk and the intensity of the oxidized flavor depending upon the quality of the hay, it was decided to arrange all values obtained in groups based on the intensity of the oxidized flavor. Figure 4 shows the relationship of ascorbic acid to intensity of oxidized flavor as shown by 580 observa-

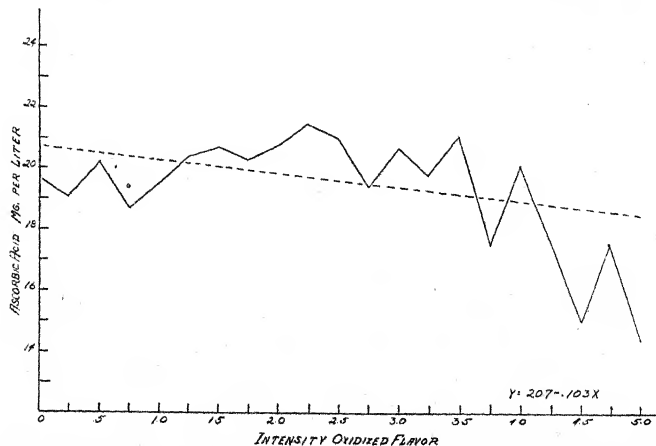


FIG. 4. The relationship between the ascorbic acid content of the milk and the intensity of metal-induced oxidized flavor.

tions. The broken line is the "line of least squares" as calculated from the individual observations. Although the slant of the line is not steep it appears that there is a tendency for milk which develops a strong degree of oxidized flavor to have a lower original ascorbic acid content. This compares favorably with results in the literature previously mentioned (9).

Carotene likewise has been claimed to be a major factor in the susceptibility of milk to oxidized flavor. It seemed desirable to plot the carotene content against the intensity of the oxidized flavor in the same manner. The results of this study are shown in figure 5 which represents the data from

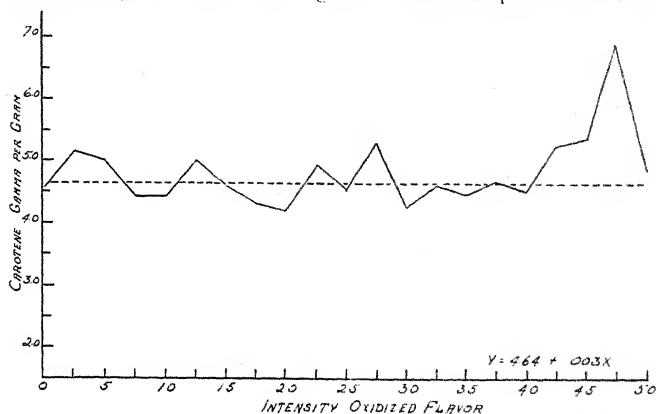


FIG. 5. The relationship between the carotene content of the butterfat of herd milk and the intensity of metal-induced oxidized flavor.

555 observations. The line of least squares as plotted through these data reveals the lack of relationship between the two variables. Since it is common knowledge that there are breed differences in the ability of cows to

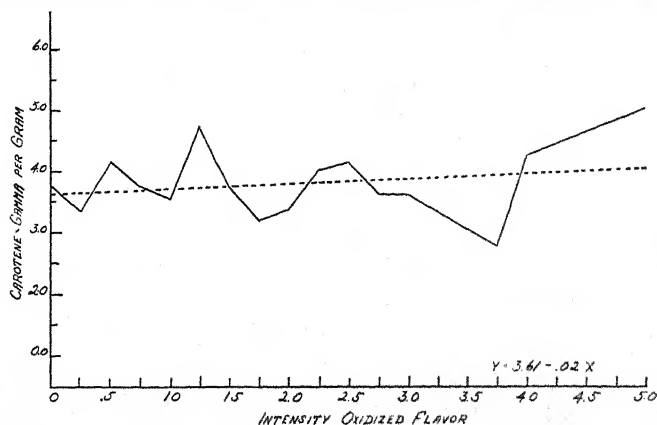


FIG. 6. The relationship between the carotene content of the butterfat of Ayrshire milk and the intensity of metal-induced oxidized flavor.

transfer carotene to the milk it was thought that this might have obscured any relationship which might have existed. Accordingly the breeds were divided into separate groups and plotted separately. These results are shown in figures 6, 7, and 8.

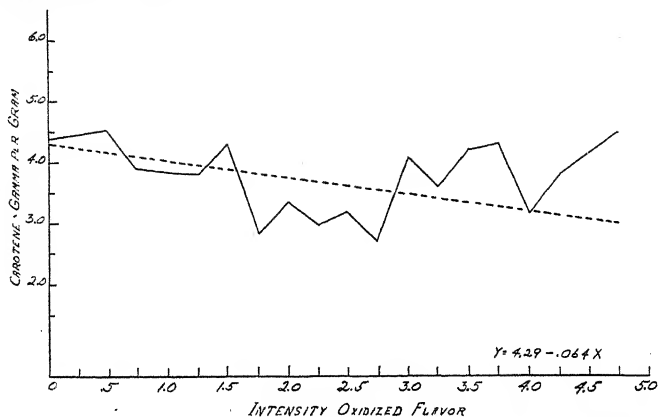


FIG. 7. The relationship between the carotene content of the butterfat of Holstein milk and the intensity of metal-induced oxidized flavor.

Figure 6 gives the results obtained for 6 Ayrshire cows with a total of 121 observations. The line of least squares shows, if anything, a direct relationship between the intensity of the oxidized flavor and the carotene content of the milk. However, in figure 7, the data obtained from 8 Holstein cows with 132 observations give a line of least squares slanting strongly in the opposite direction, indicating an inverse relationship between carotene and oxidized flavor. Figure 8, which represents 14 Jersey cows with 302 observations, is just about a median line between the two extremes. In a

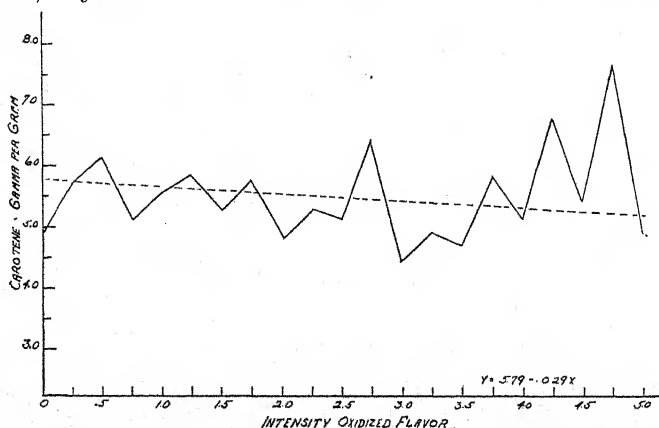


FIG. 8. The relationship between the carotene content of the butterfat of Jersey milk and the intensity of metal-induced oxidized flavor.

random distribution we would expect as many observations on one side as on the other of a horizontal line. This is the condition which exists in these data, indicating the lack of relationship between the carotene content of the milk produced on dry feed and the intensity of the metal-induced oxidized flavor.

Since all these observations were made from cows on dry feed, it is entirely possible that different results might be obtained from cows on pasture.

DISCUSSION

The results of the experiment on the quality of alfalfa hay fed to cows producing milk susceptible to oxidized flavor show that it is possible to reduce greatly or entirely eliminate the susceptibility of the milk to oxidized flavor development by feeding excellent quality alfalfa hay. The feeding of brown, leafy alfalfa hay to cows caused an appreciable decrease of the amount of carotene in the milk but did not result in an increase in the intensity of the metal-induced oxidized flavor of the milk. A summarization of these data together with those obtained from a number of cows on a normal herd ration suggests that the carotene of the milk may not be the substance responsible for the reduction in the susceptibility of the milk to oxidized flavor but that some other substance or substances associated with it in bright green hay or alfalfa leaf meal may be the important factor or factors.

Likewise, these data suggest that the ascorbic acid content of the milk may play a minor role in the susceptibility of the milk to oxidized flavor. The result of the study of nineteen cows from the time when their milk became ready for human consumption until they had been in production for three months suggests that the ascorbic acid content of the milk is low at the time of parturition and increases for a period of about seven or eight weeks after which it remains fairly constant for that particular animal. This increase apparently resulted in a slight decrease in the intensity of the oxidized flavor developed during the same period.

SUMMARY AND CONCLUSIONS

1. The feeding of high quality alfalfa hay together with alfalfa leaf meal greatly reduced or eliminated the tendency for metal-induced oxidized flavor to develop.
2. The feeding of brown, leafy alfalfa hay resulted in a decreased carotene content in the milk but did not increase the intensity of the oxidized flavor.
3. The carotene content of the milk fat at the beginning of lactation appears to be high and decreases until it reaches a normal level a few weeks after parturition.

4. The ascorbic acid content of milk at the start of lactation is usually low and increases gradually until it reaches a maximum level at about seven or eight weeks following parturition.

5. From the results obtained it appears that ascorbic acid in the milk plays a minor role in the susceptibility of the milk to metal-induced oxidized flavor.

6. The results of this study indicate that the amount of carotene in the butter fat may not be the substance responsible for the reduction in susceptibility of milk to oxidized flavor. It appears that some substance or substances associated with it probably has a greater effect than the carotene itself.

ACKNOWLEDGMENT

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A DOUBLE CHANGE-OVER DESIGN FOR DAIRY CATTLE FEEDING EXPERIMENTS*

W. G. COCHRAN, K. M. AUTREY AND C. Y. CANNON
Iowa Agricultural Experiment Station, Ames, Iowa

INTRODUCTION

During the winter of 1939-40, a feeding trial was carried out by the Iowa Agricultural Experiment Station with eighteen Holstein cows, on three planes of feeding. The three rations tested, and hereinafter designated A, B and C, respectively, were as follows:

- A. Roughage alone (alfalfa hay and corn silage).
- B. Limited grain (alfalfa hay, corn silage and grain fed at the rate of one pound for each 7 pounds of milk produced).
- C. Full grain (alfalfa hay, corn silage and grain fed at the rate of one pound for each 3.5 pounds of milk produced).

The grain mixture consisted of the following constituents in parts by weight: corn, 4; oats, 4; cracked soybeans, 1; bone meal, 0.25; and salt, 0.1.

The experiment was confined to a single lactation, and was of the switch-over type, each cow receiving each ration for a period of several weeks. The layout of the experiment was designed so as to avoid as far as possible the difficulties that commonly arise in interpreting the results of switch-over trials, and to permit tests of significance of the differences between the effects of the rations. It is hoped that a brief description of the layout and of the statistical analysis may be of interest to those engaged in dairy work.

DESCRIPTION OF THE DESIGN

In the ordinary group trial, where each cow receives only a single ration, the average milk-yield obtained for a given ration depends on the yieldability of the cows receiving the ration. Since cows are highly variable in this respect, the experimental error from this source is often large, though it may be reduced by skillful grouping of the cows. The switch-over trial represents an attempt to eliminate this source of variation entirely from the comparisons between rations, by feeding every cow in turn on all rations. However, in designing switch-over trials, other sources of experimental error must be taken into account if the maximum gain in accuracy is to be realized. One arises from the characteristic drop in milk yields towards the end of the lactation period. Thus if a cow receives rations A, B and C in succession, her total milk yields under the three rations are scarcely comparable, since A was tested during the most productive period and C during the least.

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most switch-over trials whose results we have examined, this drop in yield is a preponderating factor, the yields falling even when the poorest ration is given first and the best ration last.

This difficulty can be largely overcome by designing the experiment so that in any experimental period one-third of the cows are receiving each ration. For instance, one-third of the cows might receive the sequence ABC, one-third the sequence BCA, and one-third the sequence CAB. With this arrangement the three experimental periods are equally represented in the average milk yield for any ration. However, the effects of the lactation curve are not eliminated entirely from the experimental error of these averages unless the natural rate of fall in yield from period to period is the same for the three groups of cows. For example, if the sequence ABC is given to a group of cows whose lactation curves show high yields in the first period, thereafter dropping rapidly, whereas the other two sequences are given to cows whose lactation curves have a much smaller rate of fall, ration A is favored relatively to B and C when the average yields over all three groups are calculated. Thus the division of the available cows into three groups to receive the three sequences should not be made simply at random; instead, the object should be to obtain groups which are as similar as possible in the rates of fall of their lactation curves.

In the present experiment, the eighteen cows were first divided into six groups of three on the basis of expected yielding ability, the three cows within each group being chosen as similar as possible. The three sequences were then allotted at random to the three cows of each group. The reasoning was that cows with the same yielding ability would be likely to show a similar rate of fall in yields. This assumption appeared to be justified by the results of the trial in which this grouping was used, the total rate of fall per group decreasing markedly from the high-yielding to the low-yielding groups, as shown in table 1. In this table, as in subsequent tables, all the milk yields were converted to four per cent, fat-corrected milk (2).

The decreases in yield from period I to period II and from period II to period III were almost twice as great for the high-yielding groups as for the low-yielding groups.

TABLE 1
Total yield and rate of fall in yield of fat-corrected milk

	Groups* of three cows				
	1	2	4	5	6
Total milk yield (lbs.)	14,351	14,168	12,735	11,391	10,603
Decrease in yield (lbs.)					
Period I-II	868	889	385	521	472
Period II-III	1,019	923	598	506	495

* Group 3 omitted because one cow became sick and had to be rejected.

With this design, each group of three cows constitutes an independent experiment, of a type known as the 3×3 Latin square (fig. 1) (1).

Period	Cow		
	1	2	3
I	A	B	C
II	B	C	A
III	C	A	B

Fig. 1. The 3×3 Latin Square Design (No. 1).

A valid estimate of the experimental error may be obtained by an analysis of variance, as follows:

	Degrees of freedom
Between cows	2
Between periods	2
Between rations	2
Error	2
Total	8

Differences between cows and between periods are not included in the estimate of error, since by the nature of the design they are excluded from the true errors of the ration means. Each of the six groups provides a separate analysis of variance of the above type. On combining the six analyses, the following subdivision of degrees of freedom is obtained for the whole experiment:

	Degrees of freedom
Between groups	5
Between cows within groups	12
Between periods within groups	12
Between rations	2
Interaction of rations and groups	10
Error	12
Total	53

The 12 degrees of freedom for *between cows*, *between periods* and *error* are the totals of the corresponding terms in the individual groups. A similar set of 12 degrees of freedom is obtained for *between rations*. This may be divided into two parts: 2 degrees of freedom representing the average differences between rations over the whole experiment, and 10 degrees of freedom representing variations from group to group in the difference between the rations. If the differences between the rations are the same in all groups (apart from experimental errors), the mean square for this term should be no larger than the error mean square, and may legitimately be included in the error. However, the term should be calculated separately to test this point, particularly where the groups differ widely in yielding ability or in other respects, *e.g.*, breed. The remaining 5 degrees of freedom corresponding to differences between the group totals, are not included in the individual analyses being given here for example.

For reasons to be explained later, three of the groups were assigned to the Latin square given above, and the remaining three to the complementary square as shown in figure 2. This feature introduces no change in the method of analysis.

A B C
C A B
B C A

FIG. 2. The 3×3 Latin Square (No. 2).

ANALYSIS OF VARIANCE OF THE TOTAL DIGESTIBLE NUTRIENT CONSUMPTION

The consumption of digestible nutrients per cow for each period is given in table 2. Each of the groups in this table was analyzed separately and the degrees of freedom, as well as the sums of squares, were combined (table 3), according to the procedure previously described. The data on group 3 were omitted from table 2 due to the loss of one of the cows in it, thus causing a loss of nine degrees of freedom in the combined analysis.

TABLE 2

Individual total digestible nutrient consumption units: lbs. per cow per period (total of six weeks)

Group 1								Group 2							
Period		Cow						Period		Cow					
		1	2	3			total			4	5	6			total
I	A	608	B	885	C	940	2433	A	527	B	696	C	989		2212
II	B	715	C	1087	A	766	2568	C	883	A	635	B	899		2417
III	C	844	A	711	B	832	2387	B	785	C	901	A	657		2343
Total		2167		2683		2538	7388			2195		2232		2545	6972
Group 3								Group 4							
Period		Cow						Period		Cow					
		7	8	9						10	11	12			total
This group omitted because cow No. 7 was removed from the experiment in Period I, owing to kidney cancer.								A	472	B	734	C	897		2103
								C	819	A	644	B	766		2229
								B	778	C	953	A	706		2437
										2069		2331		2369	6769
Group 5								Group 6							
Period		Cow						Period		Cow					
		13	14	15			total			16	17	18			total
I	A	586	B	635	C	805	2026	A	489	B	593	C	788		1870
II	B	723	C	799	A	542	2064	C	730	A	536	B	695		1961
III	C	892	A	595	B	681	2168	B	674	C	758	A	609		2041
Total		2201		2029		2028	6258			1893		1887		2092	5872

A = roughage, B = limited grain, C = full grain.

TABLE 3

Analysis of variance (3) of t.d.n. consumption units: total consumption per cow per period, in lbs.

	Degrees of freedom	Sums of squares	Mean squares
1. Between groups	4	157,936	39,484†
2. Between cows within groups	10	105,336	10,534†
3. Between periods within groups ...	10	40,534	4,053*
4. Between rations	2	533,869	266,934†
5. Ration × group interactions	8	12,021	1,503
6. Error	10	14,100	1,410
7. Total	44	863,796	

* Significant.

† Highly significant.

Since the analysis of variance in table 3 shows the "ration × group interaction" to be insignificant, this term was included in the experimental error. The mean square of the ration effects (266,948) is significantly greater than the error mean square (1450), signifying that the consumption of nutrients was appreciably increased by the addition of grain to the ration. The F-test (3) reveals that the differences between groups are also highly significant, the mean square for that term being 39,490 as compared with only 1450 for the error. This shows that the arrangement of cows into outcome groups was well justified.

TABLE 4

Digestible nutrient consumption-totals and means units: lbs. for six weeks periods

Rations	Ration totals (15 cows)	Ration means	Standard error
A	9,083	605.5	
B	11,091	739.4	± 9.83
C	13,085	872.3	
Grand total =	33,259	General mean = 739.1	

Table 4 shows the mean digestible nutrient consumption for each ration, and the standard error per cow. The standard error, $\sqrt{1451} = 38.08$ is 5.15 per cent of the general mean, 739.1. The standard error for the mean of 15 cows is $38.08/\sqrt{15} = 9.83$ pounds, or only 1.33 per cent of the mean. The relative performance of the groups was in the order anticipated, all differences between pairs of rations being highly significant.

ADJUSTMENTS FOR CARRY-OVER EFFECTS

In the case of total nutrient consumption the amount eaten in one period did not appear to be influenced by the ration given in the previous period. However, with other factors, particularly total milk yield, a carry-over effect of the ration given in the previous period may be anticipated, owing to the shortness of the change-over period from one ration to another.

If such residual effects are present, simple averages do not give unbiased estimates of the effects of the rations. For instance, with the layout shown in figure 1, ration A is preceded by ration C in both the second and third periods, and likewise B by A and C by B. If A is the poorest ration and C the best, the milk yields for ration A may be increased by the beneficial carry-over effect of C, while those for ration C may be depressed by the carry-over effect of A. In these circumstances, the simple averages would *underestimate* the differences between the direct effects of the rations.

Thus when carry-over effects are present, the average milk yields under each ration must be adjusted in some way to avoid bias. These adjustments can clearly be made only if the design of the experiment enables us to estimate the sizes of the carry-over effects. This cannot be done with the design shown in figure 1, since each ration is always followed by the same ration. Thus, if this design is used for all six groups, no corrections for carry-over effects are possible, except perhaps by inspecting the daily yields, omitting from the results those parts of the experimental periods which appear to show carry-over effects. This method is at best somewhat arbitrary, and would be unsatisfactory if carry-over effects persisted through most of the succeeding periods.

However, by using both the cycles shown in figures 1 and 2, a direct evaluation of the carry-over effects is possible. This may be seen by examining the total milk yields for each of the six cycles, shown in table 5.

TABLE 5
Total milk yields for each of the six ration cycles
(Totals for 3 cows (18 weeks) in lbs.)

Period	Sets*					
	1	2	3	4	5	6
I A	4,383	B 5,370	C 5,720	A 4,427	B 4,675	C 5,236
II B	4,057	C 4,980	A 4,208	C 4,535	A 3,637	B 4,420
III C	3,834	A 3,264	B 3,571	B 3,833	C 3,787	A 2,956
Set totals	12,274	13,614	13,499	12,795	12,099	12,612
Grand total	76,893					
Ration totals:	A = Roughage		B = Limited grain		C = Full grain	
	A = 22,875		B = 25,926		C = 28,092	

* To complete set 1, in which a cow was rejected, values were inserted for the missing cow by a method described below.

The results during the first period do not require adjustment, since all cows received the same preceding ration. In the second period, two sets of cows, 3 and 5, received roughage. Of these, set 3, having had full grain in the previous period, gave a total of 4208 lbs., while set 5, having had limited grain in the previous period, gave a total of 3637 lbs. The difference, 571 lbs., is an estimate of the difference between the carry-over effect

of full grain and that of limited grain. Similarly, a comparison of the carry-over effects of full grain and roughage is obtained from sets 1 and 6 in the second period, and a comparison between limited grain and roughage from sets 2 and 4. The same comparisons are repeated with the results in the third period. In all six comparisons, the carry-over effect was in the same direction as the direct effect (*i.e.*, full grain > limited grain > roughage), indicating strongly that real carry-over effects were present.

We may now consider how to obtain estimates of the direct effects of the rations, free from the disturbance due to carry-over effects. This may be done in many ways. For instance, an unbiased comparison of the direct effects of roughage and limited grain in each period is given by the mean milk yields of the following groups of cows:

Period	Roughage	Limited grain
I	Sets 1 and 4	Sets 2 and 5
II	Set 3	Set 6
III	Set 2	Set 4

Since sets 3 and 6 both had full grain in the first period, their yields in the second period, under roughage and limited grain respectively, are subject to the same carry-over effect, and similarly for sets 2 and 4 in the third period. An estimate of the difference between the two rations for the whole experiment could be obtained by taking some simple average of the differences in the individual periods. Such an estimate is, however, open to criticism on the grounds that only half of the available cows are used in the second and third periods. A further objection, probably more serious, is that the estimate would be partly a group comparison, since only the cows in sets 2 and 4 are common to both roughage and limited grain. The use of such an estimate would therefore defeat the purpose of the switch-over design, which is to avoid group comparisons.

To retain the full advantage of the switch-over design, the estimate of the difference between the effects of two rations must clearly be unaffected by differences in the yielding ability of the cows or by differences between the average yields for different periods. The most accurate estimates, subject to these restrictions, are given by a technique known as the method of least squares, which is frequently used for dealing with similar problems arising in field experiments. In this procedure, the observed milk yield for any cow in any period is expressed as a linear function of the effects of the cow's yielding ability, the period, the current and previous rations, and the experimental error. For example, consider a cow receiving in succession limited grain, full grain and roughage. Its milk yields y_1 , y_2 , y_3 in the three periods are expressed as follows:

$$y_1 = m + p_1 + d_b + e_1$$

$$y_2 = m + p_2 + d_c + r_b + e_2$$

$$y_3 = m + p_3 + d_a + r_c + e_3$$

where m = mean yield for the cow

p_1, p_2, p_3 = effects of the three periods

d_a, d_b, d_c = direct effects of the rations

r_1, r_c = residual effects of the rations given in the previous period

e_1, e_2, e_3 = experimental errors.

No parameter is required to represent residual effects in the first period. The constants p_1, p_2, p_3 remain unchanged for all three cows in the same group, since only the group averages of the differences between periods are eliminated from the experimental errors. After setting up equations of this type for every cow, the constants are estimated by minimizing the sum of squares of the experimental errors. The details of the mathematical analysis will not be given here. To calculate the adjusted yields given by the least squares solution, the following quantities (Table 6) are required, all being easily obtainable from table 5.

TABLE 6
Data required for calculating the adjusted yields for each ration

A	22,875	a	15,950	$s_2 + s_3$	26,226		
B	25,926	b	15,407	$s_3 + s_4$	26,294	T	76,893
C	28,092	c	15,725	$s_1 + s_5$	24,373	(2/3)T	51,262

Here A, B, C are simply the total yields under the three rations, as given at the foot of table 5. The quantities a, b, c are the total yields of all cows receiving these respective rations in the previous period.

e.g., $a = 4057 + 3571 + 4535 + 3787 = 15,950$, etc.

Also $s_2 + s_3$ = total of sets 2 and 6 = $13,614 + 12,612 = 26,226$, etc.¹ Finally, we required two-thirds of the total of all yields in table 6 [$(\frac{2}{3})T = 51,262$]. The adjusted mean yields per cow per period (total of six weeks) are then given by the following equations:

Roughage

$$\begin{aligned} 72\bar{y}_a &= 5A + (2a - b - c) + s_2 + s_6 - (\tfrac{2}{3})T \\ &= 5 \times 22,875 + (2 \times 15,950 - 15,407 - 15,725) + 26,226 - 51,262 \\ &= 90,107 \end{aligned}$$

Hence $\bar{y}_a = 1251.5$

Limited grain

$$72\bar{y}_b = 5B + (2b - a - c) - s_3 + s_4 - (\tfrac{2}{3})T = 103,801 \text{ giving } \bar{y}_b = 1441.7$$

Full grain

$$72\bar{y}_c = 5C + (2c - a - b) + s_1 + s_5 - (\tfrac{2}{3})T = 113,664 \text{ giving } \bar{y}_c = 1578.7$$

The corresponding unadjusted mean yields per cow per period are the totals A, B and C, divided by 18 (the number of cows in each total).

The adjustments reduced the mean yield under roughage by 19 lbs., and increased the mean yield under full grain by about the same amount, these

¹Sets 2 and 6 received roughage during period III; Sets 3 and 4 received limited grain, and Sets 1 and 5 full grain.

TABLE 7

Mean yields of fat corrected milk per cow per period of six weeks

Ration	Unadjusted			Adjusted for carry-over effects		
	Mean	Increase over roughage		Mean	Increase over roughage	
	<i>lbs.</i>	<i>lbs.</i>	<i>%</i>	<i>lbs.</i>	<i>lbs.</i>	<i>%</i>
A	1270.8			1251.5		
B	1440.3	+ 169.5	+ 13.3	1441.7	+ 190.2	+ 15.2
C	1560.7	+ 289.9	+ 22.8	1578.7	+ 327.2	+ 26.1
Standard error \pm				21.4	\pm 30.3	\pm 2.4

effects being in the direction anticipated by the previous discussion. It is interesting to observe that the mean yield is practically unaltered for the cows receiving limited grain, for which the beneficial carry-over effect from full grain and the detrimental effect from roughage appeared to cancel. By failure to adjust for carry-over effects, the differences between the rations would have been underestimated by about 11 per cent.

To verify that the yields have been freed from carry-over effects, we may create artificially an additional carry-over effect of say, the full grain ration, by adding 1000 to the yields in table 5, wherever full grain was fed in the previous period. The yields affected are 3264 (set 2), 4208 (set 3), 3833 (set 4) and 4420 (set 6). With this change, the reader may verify that the adjusted mean yields become:

Roughage 1325.6 lbs., Limited grain 1515.8 lbs., Full grain 1652.7 lbs.

The effect of the artificial carry-over effect is simply to increase all three means by the same amount, 74.1 lbs., the differences between the ration means remaining exactly the same as before. With unadjusted means, on the other hand, the yields for roughage and limited grain would both be increased by 111.1 lbs., that for full grain being unchanged. The same property holds for the adjusted mean yields if a constant amount is added to all yields following any one of the three rations.

It may be of interest to examine the differences between the carry-over effects of the three rations. For a single cow (total of six weeks), these are given by the deviations of the following quantities (Table 8) from their mean.

TABLE 8

Estimates of the carry-over effects per cow (total for 6 weeks)

		Deviation (lbs.)
Roughage:	$(3a + A + s_2 + s_0)/24 = 96,951/24 = 4,040$	- 58
Limited grain:	$(3b + B + s_3 + s_4)/24 = 98,441/24 = 4,102$	+ 4
Full grain:	$(3c + C + s_1 + s_5)/24 = 99,640/24 = 4,152$	+ 54

The difference in carry-over effect between roughage and full grain was 112 lbs., about one-third of the corresponding difference (327 lbs.) between the direct effects. The same ratio holds approximately for the other two differences between the rations. The carry-over effects seem strikingly large in relation to the direct effects; however, the figures given above agree well with the rough estimates which may be made from table 5. The six cows receiving limited grain during the second and third periods and full grain in the previous period gave a total yield of $4420 + 3833 = 8253$ lbs., as compared with 7628 lbs. for the six cows having limited grain preceded by roughage. The difference, 625 lbs., corresponds to 104 lbs. per cow, which checks closely with the estimate of 112 lbs. given above.

The adjustments required for the direct effects may be obtained from the above estimates for the carry-over effects. For example, of the eighteen yields from cows receiving roughage, six followed a previous ration of limited grain, six followed full grain, while none followed roughage. Thus the total yield of the eighteen cows should be reduced by six times the sum of the carry-over effects for limited grain and roughage, *i.e.*, the mean should be reduced by

$$(6, 18) [54 + 4] = 58/3 = 19.3 \text{ lbs.};$$

in agreement with the reduction already given in table 7.

THE ANALYSIS OF VARIANCE WHEN CARRY-OVER EFFECTS ARE PRESENT

In analyzing the results for total nutrient consumption, we discarded results for the group containing the cow which was removed from the experiment. While this is the simplest procedure, it results in an appreciable loss of information. By a technique described by Yates (4) it is possible to include in the analysis the two healthy cows in group 3. The first step in this technique is to estimate results for the missing cow by the method of least squares. The completed data can then be analyzed by the usual procedure, with certain slight changes mentioned below.

The individual yields of fat corrected milk are shown in table 9, the values inserted for the missing cow being denoted by asterisks. In the analysis of variance, the total sum of squares and the sums of squares for groups, cows within groups, periods and the periods by groups interaction are found in the same way as for total nutrient consumption. Only the sums of squares between rations require special consideration. Of these, two degrees of freedom measure differences between the direct effects of the three rations, and two measure differences between the carry-over effects. However, the two sets of two degrees of freedom do not add to the correct total sum of squares for direct and carry-over effects, because the two effects are entangled by the nature of the design. This total sum of squares (4 degrees of freedom) may be obtained either as direct effects (ignoring carry-over effects) + carry-over effects, or as carry-over effects (ignoring direct effects) + direct effects.

TABLE 9

Individual yields of fat corrected milk units: lbs. per cow per period (total of six weeks)

Group 1							Group 2							
Period	Cow						Totals	Cow						Totals
	1	2	3	4	5	6								
I	A	1376	B	2088	C	2238	5702	A	1863	B	1748	C	2012	5623
II	B	1246	C	1864	A	1724	4834	C	1755	A	1353	B	1626	4734
III	C	1151	A	1392	B	1272	3815	B	1462	C	1339	A	1010	3811
Totals		3773		5344		5234	14351		5080		4440		4648	14168
Group 3							Group 4							
Period	Cow						Totals	Cow						Totals
	7	8	9	10	11	12								
I	A	1665*	B	1938	C	1855	5458	A	1384	B	1640	C	1677	4701
II	B	1517*	C	1804	A	1298	4619	C	1535	A	1284	B	1497	4316
III	C	1366*	A	969	B	1233	3568	B	1289	C	1370	A	1059	3718
Totals		4548*		4711		4386	13645		4208		4294		4233	12735
Group 5							Group 6							
Period	Cow						Totals	Cow						Totals
	13	14	15	16	17	18								
I	A	1342	B	1344	C	1627	4313	A	1180	B	1287	C	1547	4014
II	B	1294	C	1312	A	1186	3792	C	1245	A	1000	B	1297	3542
III	C	1317	A	903	B	1066	3286	B	1082	C	1078	A	887	3047
Totals		3953		3559		3879	11391		3507		3365		3731	10603

A = Roughage, B = Limited grain, C = Full grain.

* Values inserted for cow which was rejected during the first period.

The sum of squares for direct effects (ignoring carry-over effects) is found in the same way as for total nutrient consumption, being:

$$(1/18) [(22,875)^2 + (25,926)^2 + (28,092)^2 - 1/3 (76,893)^2] = 763,282.$$

The sum of squares for direct effects (adjusted for carry-over effects) is obtained from the adjusted ration totals given above (p. 944), as follows:

$$(1/360) [(90,107)^2 + (103,801)^2 + (113,664)^2 - 1/3 (307,572)^2] = 777,534.$$

To calculate the two sums of squares for carry-over effects, we require the totals for the three rations, with and without adjustment for direct effects. The former have already been obtained (Table 8).

The adjusted sum of squares is:

$$(1/72) [(96,951)^2 + (98,441)^2 + (99,640)^2 - 1/3 (295,032)^2] = 50,409.$$

The unadjusted totals are given by subtracting A, B and C, respectively from the corresponding adjusted totals. The sum of squares is:

$$(1/90) [(74,076)^2 + (72,515)^2 + (71,548)^2 - 1/3 (218,139)^2] = 36,158.$$

As a check, we may note that:

$$763,282 + 50,409 = 813,691 : 777,534 + 36,158 = 813,692;$$

this figure being the total sum of squares (4 degrees of freedom) for direct and residual effects. The error sum of squares is found by subtraction.

TABLE 10

Analysis of variance of fat corrected milk units: total yield per cow per period, in lbs.

	Degrees of freedom	Sum of squares	Mean squares
Total	50	5,124,267	
Periods	2	2,041,769	
Interaction of periods with groups	10	193,341	19,334
Groups	5	1,311,769	262,354
Cows within groups	11*	654,638	59,513
{ Residual (ignoring direct)	2	36,158	388,767
{ Direct	2	777,534	
Rotations { Direct (ignoring residual)	2	763,282	
{ Residual	2	50,409	25,204
Error	18†	109,058	6,059

* One degree of freedom subtracted for missing cow.

† Two degrees of freedom subtracted for missing cow.

The complete analysis of variance is shown in table 10, both sets of 2 degrees of freedom for direct and carry-over effects being included. While only one set need be calculated to obtain the error sum of squares by subtraction, both are required if tests of significance of the direct and carry-over effects are desired. The mean squares for direct effects (388,767) and carry-over effects (25,204), are both significant, the 5 per cent F-value for 2 and 18 degrees of freedom being 3.55. The estimated standard error per cow (total of six weeks) is 77.84 lbs., or 5.5 per cent of the mean yield of 1424 lbs. The corresponding standard error for the mean of 18 cows would be $77.84/\sqrt{18}$. However, this must be increased to allow for the missing cow, and for the adjustments necessary to correct for carry-over effects. The effect of the missing cow is to decrease the effective replication from 18 to nearly 16.5, while the adjustments increase the standard error by the factor $\sqrt{1.25}$. Thus the standard error of the adjusted mean yields in table 7 is $77.84 \times \sqrt{1.25}/\sqrt{16.5} = 21.4$ lbs., or 1.5 per cent of the mean. The design attained a satisfactory degree of precision; an observed increase of 5 per cent would have been detected as statistically significant, while the actual differences between the ration means were all highly significant.

It has already been noticed (table 1) that the rates of fall in yield from period I to period III differed greatly for the different groups of cows. This fact is reflected in the analysis of variance, the mean square for the interaction of periods with groups being 19,334 as against 6,059 for the error mean square. If the six cycles had been assigned to the eighteen cows at random, with three cows to each cycle, the estimated error mean square would have been approximately $(24 \times 6,059 + 10 \times 19,334)/34 = 9,963$. The device of first dividing the cows into six groups of three on the basis of expected yielding ability therefore resulted in a marked increase in precision.

Similarly, the mean square between cows in the same group (59,513) is much larger than the error mean square. This indicates that any estimates based on group comparisons would have been subject to much higher experimental errors than those avoiding the use of group comparisons.

There is, of course, nothing to be gained by adjusting the means in cases where there are no carry-over effects. The important feature of the design is that it enables corrections to be made where these are considered necessary. An examination of the records, presented as in table 5 will usually be sufficient to decide the issue. In more doubtful cases, the direct and residual effects should be calculated as above, comparing the mean square for residual effects with the error mean square in the analysis of variance. If the former mean square is noticeably higher than the latter, it is safer to make the adjustments.

OTHER NUMBERS OF REPLICATIONS AND TREATMENTS

In the above design, the experimental unit consists of six cows, one receiving each cycle. To retain the full balance of the design, the number of cows used must be a multiple of six. In an experiment with $6k$ cows, the calculations are exactly as described above, except that all divisors should be multiplied by $k/3$. Thus if 24 cows had been used, the expressions $72\bar{y}_a$, $72\bar{y}_b$, $72\bar{y}_c$ for the adjusted totals would be replaced by $96\bar{y}_a$, $96\bar{y}_b$, $96\bar{y}_c$ respectively, the righthand sides of the equations remaining unchanged. The same rule applies to the divisors in the analysis of variance. Where there are no missing cows, the standard error of the adjusted means is

$$\sqrt{\frac{1.25s^2}{6k}} \text{ where } s^2 \text{ is the error mean square in the analysis of variance.}$$

Adjustments of this type cannot be made when only two rations are being compared in a switch-over design, since each ration must always precede the other. To obtain direct information on carry-over effects, say in a single reversal trial, it would be necessary to include cows receiving the sequences AA and BB, as well as cows receiving the switch-over sequences AB and BA.

With four treatments there are twenty-four possible cycles. However, the important features of the design can be obtained with only twelve cows, by the use of the following trio of 4×4 Latin squares:

Period	Cows				Cows				Cows			
	1	2	3	4	5	6	7	8	9	10	11	12
I	A	B	C	D	A	B	C	D	A	B	C	D
II	B	A	D	C	D	C	B	A	C	D	A	B
III	C	D	A	B	B	A	D	C	D	C	B	A
IV	D	C	B	A	C	D	A	B	B	A	D	C

FIG. 3. Change-over design for four rations.

Of the three A's in period II, one follows B, one follows C and one follows D. The same is true for any ration in any period after the first. The quantities required for estimating the direct and residual effects of ration A (with and without adjustment) are shown below.

TABLE 11
Calculation of the direct and residual effects in an experiment with four rations

Effect	Totals	Divisors	
		For means	For analysis of variance
Direct (unadjusted) ...	A	12	12
Direct (adjusted)	$11A + (3a - b - c - d) + s_1 + s_7 + s_{10} - \frac{1}{2} T$	120	1320
Residual (unadjusted) ...	$4a + s_1 + s_7 + s_{10}$	132
Residual (adjusted) ...	$4a + A + s_1 + s_7 + s_{10}$	30	120

The symbols here have the same definitions as on pages 14 and 15. The sets s_1, s_7, s_{10} are those in which A is given during the fourth period, the same rule applying to rations B, C and D. The first column of divisors gives the numbers by which the corresponding totals must be divided to obtain estimates per cow per period. The second column shows the divisors required in the analysis of variance; in each case these apply to the sums of squares of deviations of the totals for the four rations from their mean. Since the divisors are for a single unit of 12 cows, they must be multiplied by k where k units are used in the experiment. The formula for the standard error of the adjusted means per cow per period is $\sqrt{\frac{1.1s^2}{12k}}$, where s^2 is the error mean square.

Since four is probably the maximum number of rations that could be given in a single lactation, no designs are presented for higher numbers of rations.

It should be understood that the designs described above apply particularly to short-time trials. Since it is difficult to keep a group of cows going for several lactations without some losses, such designs would require modification for use in long-time trials (of 3-4 years). Animal losses in experiments of this type destroy the balance of the design, thus making a thorough and efficient analysis of the results difficult.

SUMMARY

The design and statistical analysis of a short-time switch-over trial comparing three rations are discussed in this paper. The principal objects of the design are to secure accurate comparisons of the effects of the rations and unbiased estimates of the experimental errors. The design also makes it possible to estimate and adjust for carry-over effects, which have usually

been ignored in switch-over trials. The appropriate statistical analyses are illustrated by numerical examples of the case in which carry-over effects are negligible (nutrient consumption) and the case in which they are not negligible (milk production). A corresponding design for four rations is briefly described.

A detailed report of the results of this feeding trial will be given in a future paper.

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THE EFFECT OF PROCESSING ON THE NITROGEN DISTRIBUTION IN MILK

S. G. MENEFFEE, O. R. OVERMAN AND P. H. TRACY

Department of Dairy Husbandry, University of Illinois, Urbana, Illinois

Knowledge of the changes that take place in the proteins when milk is heated is obscure and most studies that pertain to this subject have been confined largely to the amount of coagulation that occurs at different temperatures. Considerable work has been done in this field but the results as a whole are difficult to compare because different temperature-time relationships and various analytical methods have been used to study the N distribution. Such studies also are complicated by the variable composition of milk which has a direct influence on the stability of the milk proteins when they are subjected to heat.

In most studies concerning the N distribution in milk macro-Kjeldahl procedures have been used which necessitate the filtration and transfer of bulky precipitates. Furthermore large amounts of reagents must be used to separate certain protein fractions and consequently large volumes of filtrate result which must be used in the subsequent analyses.

The macro procedures for determining the N fractions of milk are tedious and time consuming and limit the amount of experimental work that may be accomplished in a given time. Since previous experiments (1) showed that a semimicro procedure was as accurate as the macro methods for the determination of total N in milk it seemed probable that the separation and analysis of the protein fractions of this product could be made by semimicro methods.

This investigation was conducted in order to (a) make a preliminary study of the effect of processing on the N distribution in some of the more common milk products and (b) determine the routine applicability of a semimicro method recommended by Rowland (2) for separating the protein fractions of milk.

PROCEDURE

The milk samples used in this investigation were taken from the milk delivered to the University Creamery. Mixed herd milk was used for these experiments because it would tend to be of more uniform composition and this would introduce less error in the comparisons of the data in the various tables.

All samples were taken at the indicated stages of processing and analyzed, in duplicate, as rapidly as possible after their preparation. Those

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not being analyzed were stored at 40° F. until needed, removed and brought to room temperature by immersion in a warm water bath.

No preservatives were added to the samples and none were stored long enough for acidity to develop as a result of bacterial action.

After warming the milk to room temperature it was weighed with Mojonnier weighing crosses and pipets and the pipets emptied into volumetric flasks of the capacity indicated in the following procedure recommended (2) for separating the protein fractions of milk. (If desired, samples may be weighed directly into the volumetric flasks.)

SEPARATION OF THE PROTEIN FRACTIONS OF MILK

I. *Total Nitrogen*

Weigh 5 gm. of milk into a 100-ml. flask. Dilute to the mark with water, mix, and pipet 20 ml. into a 300-ml. Kjeldahl digestion flask.

II. *Non-Casein N*

Weigh 10 gm. of milk into a 100-ml. flask, add 70–80 ml. of water at 40° C. and 1 ml. of 10 per cent acetic acid solution. Mix and after 10 minutes add 1 ml. of normal sodium acetate¹ and mix again. Cool and make to the mark with water. Filter through quantitative paper and pipet 20 ml. of the filtrate into a 300 ml. Kjeldahl flask.

The non-casein N determined this way is reduced to correct for the volume occupied in the 100 ml. flask by the precipitate of casein and fat (0.5 ml.). The correction factor (2) is non-casein N \times 0.995. In all analyses this factor has been used regardless of variation in the fat and casein contents of the milk and neglecting this variation introduces extremely small errors in the non-casein and the casein N values.

III. *Non-Protein N*

Weigh 10 gm. of milk into a 50-ml. flask. Dilute to the mark with 15 per cent trichloroacetic acid solution and mix immediately. Filter and pipet 20 ml. of the filtrate into a 300-ml. Kjeldahl flask.

IV. *Proteose-Peptide plus Non-Protein N*

Weigh 10 gm. of milk into a 100-ml. flask. Heat for 20 minutes at 95° C. and precipitate the denatured albumin and globulin with the casein as in II (non-casein N). Filter, and pipet 20 ml. of the filtrate into a 300-ml. Kjeldahl digestion flask.

V. *Globulin N*

Pipet 20 ml. of the filtrate from II (non-casein N) into a 50-ml. beaker. Add brom thymol blue indicator and 0.1 N sodium hydroxide until color

¹ Mold growth in the sodium acetate may be prevented by adding a few drops of chloroform.

shows a pH range within 6.8 to 7.2. Saturate with magnesium sulphate—about 21 gms. Set beaker aside at room temperature for at least 4 hours. Filter (suction—5-cm. filter) and wash with saturated magnesium sulphate solution. Transfer precipitate and filter paper to a 300-ml. Kjeldahl flask.

A semimicro-Kjeldahl method (1) was used to determine the N in the various protein fractions. Mercuric oxide was used as the catalyst and boric acid as the ammonia receiving agent.

Five direct determinations of the nitrogen fractions of milk are made by these methods: I—Total N; II—Non-casein N; III—Non-protein N; IV—Proteose-Peptone plus Non-protein N; V—Globulin N.

Other nitrogen fractions are determined by difference: Casein N = I-II; Albumin plus globulin N = II-IV; Albumin N = II-(IV plus V); Proteose-Peptone N = IV-III.

ANALYTICAL RESULTS

The N distribution in milk and the various milk products is tabulated as per cent nitrogen and the nitrogen in the various fractions is calculated as percentage of the total nitrogen.

Nitrogen Distribution in Homogenized Milk. Homogenized milk was analyzed for nitrogen distribution to study the effect of homogenization on the milk proteins.

TABLE 1

Nitrogen distribution in 4 per cent milk homogenized at 2500 lbs. pressure

Control—pasteurized whole milk containing 4 per cent fat. Some of the same milk, after pasteurization, was cooled to 135° F., homogenized with single stage at 2500 lbs. pressure and cooled to 40° F.

	Past.† control (per cent N)	Per cent of total N	Homo. 2500 lbs. (per cent N)	Per cent of total N
Total N	0.5408		0.5362	
Non-casein N	0.1106	20.45	0.1090	20.33
Non-protein N.....	0.0301	5.58	0.0294	5.48
Globulin N	0.0187	3.46	0.0174	3.25
Casein N	0.4302	79.55	0.4272	79.67
Albumin N	0.0434	8.03	0.0495	9.23
Proteose N*	0.0184	3.40	0.0127	2.37

* In these data proteose N is meant to include proteose-peptone N.

† Pasteurization, where indicated, means that the product has been heated for thirty minutes at 145° F. and cooled to 40° F.

In tables 2 and 3 the data reported were obtained by the analysis of 4 per cent whole milk processed the same as the product in table 1 except the milk was homogenized at a higher pressure.

In another experiment the fat content of the whole milk was increased to 8 per cent and the sample processed the same as the product referred to in table 1 except the milk was homogenized at 4000 lbs. pressure. The results are given in table 4.

TABLE 2

Nitrogen distribution in 4 per cent milk homogenized at 3500 lbs. pressure

	Past. control (per cent N)	Per cent of total N	Homo. 3500 lbs. (per cent N)	Per cent of total N
Total N	0.5288		0.5216	
Non-casein N	0.1015	19.19	0.0915	17.54
Non-protein N	0.0248	4.69	0.0252	4.83
Globulin N	0.0193	3.65	0.0093	1.78
Casein N	0.4273	80.81	0.4301	82.46
Albumin N	0.0414	7.83	0.0453	8.68
Proteose N	0.0160	3.03	0.0117	2.24

TABLE 3

Nitrogen distribution in 4 per cent milk homogenized at 5000 lbs. pressure

	Raw (per cent N)	Clarified at 70° F. (per cent N)	Past. (per cent N)	Homo. 5000 lbs. (per cent N)
Total N	0.5477	0.5611	0.5562	0.5356
Non-casein N	0.1147	0.1203	0.1143	0.1076
Non-protein N	0.0303	0.0323	0.0287	0.0282
Globulin N	0.0161	0.0185	0.0162	0.0145
Casein N	0.4330	0.4408	0.4419	0.4280
Albumin N	0.0537	0.0545	0.0510	0.0473
Proteose N	0.0146	0.0150	0.0184	0.0176

Data above calculated as percentage of the total nitrogen

Non-casein N	20.94	21.44	20.55	20.09
Non-protein N	5.53	5.76	5.16	5.27
Globulin N	2.94	3.30	2.91	2.71
Casein N	79.24	78.56	79.45	79.91
Albumin N	9.80	9.71	9.17	8.83
Proteose N	2.67	2.67	3.31	3.29

TABLE 4

Nitrogen distribution in 8 per cent milk homogenized at 4000 lbs. pressure

	Past. control (per cent N)	Per cent of total N	Homo. 4000 lbs. (per cent N)	Per cent of total N
Total N	0.5052		0.5111	
Non-casein N	0.1094	21.66	0.0985	19.27
Non-protein N	0.0300	5.94	0.0313	6.12
Globulin N	0.0200	3.96	0.0212	4.15
Casein N	0.3958	78.35	0.4126	80.73
Albumin N	0.0442	8.75	0.0354	6.93
Proteose N	0.0152	3.01	0.0106	2.07

The N distribution in homogenized skim milk (table 5) and homogenized 4 per cent raw milk (table 6) was studied to make comparisons with the previous tables (1-4). The skim milk was processed the same as the product referred to in table 1 and the raw milk was homogenized cold at 2500 lbs. pressure with single stage.

TABLE 5

Nitrogen distribution in skim milk homogenized at 3500 lbs. pressure

	Past. control (per cent N)	Per cent of total N	Homo. 3500 lbs. (per cent N)	Per cent of total N
Total N	0.5204		0.5207	
Non-casein N	0.1099	21.12	0.1093	20.99
Non-protein N	0.0290	5.57	0.0295	5.67
Globulin N	0.0182	3.50	0.0152	2.92
Casein N	0.4105	78.88	0.4114	79.01
Albumin N	0.0436	8.38	0.0466	8.95
Proteose N	0.0191	3.67	0.0180	3.46

TABLE 6

Nitrogen distribution in raw milk homogenized at 2500 lbs. pressure

	Raw (per cent N)	Per cent of total N	Homo. 2500 lbs.	Per cent of total N
Total N	0.4861		0.4848	
Non-casein N	0.1109	22.81	0.1111	22.92
Non-protein N	0.0289	5.95	0.0299	6.19
Globulin N	0.0178	3.66	0.0178	3.67
Casein N	0.3752	77.19	0.3737	77.08
Albumin N	0.0489	10.06	0.0504	10.40
Proteose N	0.0153	3.15	0.0130	2.68

Nitrogen Distribution in Evaporated Milk. Table 7 shows the N distribution in evaporated milk. The samples were taken during the various stages of the condensing process, as indicated in table 7, and analyzed for N distribution. The milk was condensed from 12.86 to 26.36 per cent total solids and 3.9 to 7.81 per cent fat. The condensed milk (D) after homogenization was canned and sterilized. No salts were added to the milk before sterilization. The raw, pasteurized and forewarmed milks were analyzed without any change while the condensed products were diluted with distilled water in order to bring the total solids concentration to 12.86 so that comparisons in N distribution could be made with the raw milk.

The data in table 7 show that significant changes have taken place in the protein fractions. Lower values are reported for the N fractions in the forewarmed sample but this is due to dilution of the milk with the steam condensate during the forewarming.

By the time the forewarming is completed all the albumin has been coagulated and this is determined (co-precipitated with casein at a pH of 4.6) with the casein N causing high results for this fraction. These changes are more significant in all samples of the condensed milk (C), (D) and (E).

It is interesting to compare the N distribution in the raw milk with that of the condensed milk (C). The casein N in (C) has increased 0.0753 per cent as a result of the heating while the non-casein N decreased 0.0749 per cent showing that the proteins (coagulated albumin and globulin) have

TABLE 7
Nitrogen distribution in evaporated milk

	Raw (per cent N)	Past. (per cent N)	Forewarmed 203° F. (per cent N)	*Condensed (C) (per cent N)	*Condensed (D) Homo. 2000 lbs. (per cent N)	*Condensed (E) Homo. 2000 lbs. Sterilized 240° F.-15'' (per cent N)
Total N	0.5333	0.5390	0.5026	0.5337	0.5380	0.5278
Non-casein N	0.1181	0.1128	0.0450	0.0432	0.0439	0.0393
Non-protein N	0.0297	0.0310	0.0282	0.0301	0.0309	0.0348
Globulin N	0.0139	0.0109	0.0119	0.0066	0.0075	0.0137
Casein N	0.4152	0.4262	0.4576	0.4905	0.4941	0.4685
Albumin N	0.0573	0.0530	0.0000	0.0000	0.0000	0.0000
Proteose N	0.0172	0.0179	0.0164	0.0158	0.0146	0.0187
Data above calculated as percentage of total nitrogen						
Non-casein N	22.15	20.93	8.95	8.09	8.16	11.24
Non-protein N	5.57	5.75	5.62	5.64	5.74	6.59
Globulin N	2.61	2.02	2.37	1.24	1.39	2.60
Casein N	77.85	79.07	91.05	91.91	91.84	88.76
Albumin N	10.74	9.83	00.00	00.00	00.00	00.00
Proteose N	3.23	3.32	3.26	2.96	2.71	3.54

* Diluted with distilled water to bring the total solids concentration to 12.86 or the T. S. concentration of the raw milk.

been removed from the non-casein or soluble protein fraction. The total albumin and globulin N present in the raw milk amounts to 0.0712 per cent while the condensed milk (C) shows no albumin and 0.0066 per cent globulin N indicating that the increase in casein N accounts for slightly more than the amount of albumin and globulin coagulated. The albumin and globulin coagulated amount to 0.0646 per cent N and the difference between this value and the increase in casein N (0.0753) is only 0.0107 per cent N which is within the limits of experimental error.

A comparison of the casein N fraction of the condensed milk (C) with the final product (E) would indicate that some hydrolysis of this fraction has taken place.

Nitrogen Distribution in Condensed Skim Milk. Since evaporated milk showed variations in the N distribution it seemed advisable to study the effect of the condensing process on the protein fractions of skim milk.

Pasteurized skim milk was forewarmed to 150° F. and condensed from 9.20 to 31.37 per cent total solids. The condensed samples were diluted with distilled water before analysis to bring the total solids concentration back to that of the original skim milk. The N distribution of this product is shown in table 8.

TABLE 8
Nitrogen distribution in condensed skim milk

	Past. skim (per cent N)	Forewarmed 150° F. (per cent N)	Condensed (per cent N)
Total N	0.5367	0.5395	0.5453
Non-casein N	0.1118	0.1016	0.1086
Non-protein N	0.0293	0.0291	0.0305
Globulin N	0.0143	0.0150	0.0153
Casein N	0.4249	0.4379	0.4367
Albumin N	0.0464	0.0395	0.0396
Proteose N	0.0218	0.0180	0.0232
Data above calculated as percentage of total N			
Non-casein N	20.83	18.83	19.92
Non-protein N	5.46	5.39	5.59
Globulin N	2.66	2.78	2.81
Casein N	79.17	81.17	80.08
Albumin N	8.65	7.32	7.26
Proteose N	4.06	3.34	4.25

The changes in the protein fractions in table 8 are very small. Regardless of these minor changes there is evidence to show that the value for the casein N fraction has increased due to the co-precipitation of coagulated protein which appears to be albumin. The analysis of the data in table 7 supports this trend of thought.

Nitrogen Distribution in Cultured Milk. An analysis of cultured milk is shown in table 9. Pasteurized skim milk was repasteurized for 30 minutes

at 190° F., cooled to 70° F. and starter added (1 quart of starter to 25 gallons of milk). The milk was allowed to stand overnight and the following morning it was thoroughly mixed and a sample taken for analysis. The cultured milk was analyzed for nitrogen distribution to check the efficiency of the buffered solutions used in the determinations of non-casein N and proteose-peptone plus non-protein nitrogen. This seemed to be a practical method to determine the effect of developed acidity on the separation of these protein fractions. The acidity of the repasteurized milk was 0.14 and that of the cultured milk was 0.79 per cent.

TABLE 9
Nitrogen distribution in cultured skim milk

	Past. skim 145° F. (per cent N)	Past. skim 190° F. (per cent N)	Cultured (per cent N)
Total N	0.5203	0.5275	0.5233
Non-casein N	0.1155	0.0542	0.0694
Non-protein N	0.0317	0.0412	0.0479
Globulin N	0.0231	0.0167	0.0213
Casein N	0.4048	0.4733	0.4539
Albumin N	0.0424	0.0000	0.0000
Proteose N	0.0183	0.0156	0.0264
Data above calculated as percentage of total N			
Non-casein N	22.20	10.27	13.26
Non-protein N	6.09	7.81	9.15
Globulin N	4.44	3.17	4.07
Casein N	77.80	89.73	86.74
Albumin N	8.15	00.00	00.00
Proteose N	3.52	2.96	5.04

Repasteurization of the skim milk at 190° F. has coagulated all of the albumin and probably some globulin as indicated by the apparent increase in casein N in this sample. The decrease in the casein N in the cultured milk sample is attributed to a slight hydrolysis of this fraction. By comparing this sample with the enzyme treated milks (table 10) one will observe that the changes in N distribution are less significant but are of a similar nature.

The buffers used (sodium acetate and acetic acid) seem to function rather efficiently in separating the indicated protein fractions in the cultured milk regardless of the high acidity.

Several samples of skim milk which were sealed in tin cans, sterilized and cultured with a minimum amount of starter showed a N distribution comparable to the data in table 9.

Nitrogen Distribution in Enzyme Treated Milks. Four per cent raw milk was heated to 145° F. in glass stoppered bottles and then Enzylac, Trypsin and Steapsin added in a concentration of 1 part of enzyme to 25,000 parts of milk. The pasteurization process was completed and all samples analyzed for N distribution. The results are shown in table 10.

TABLE 10
Nitrogen distribution in enzyme treated milks

	Control Past. 145°-30'' (per cent N)	Steapsin at 145°-Past. (per cent N)	Trypsin at 145°-Past. (per cent N)	Enzylac at 145°-Past. (per cent N)
Total N	0.5194	0.5327	0.5286	0.5138
Non-casein N	0.1098	0.1365	0.1492	0.1326
Non-protein N	0.0335	0.0419	0.0531	0.0414
Casein N	0.4096	0.3962	0.3794	0.3812
Alb. and Glob. N*	0.0523	0.0521	0.0657	0.0483
Proteose N	0.0240	0.0425	0.0304	0.0429
Data above calculated as percentage of total N				
Non-casein N	21.14	25.62	28.23	25.81
Non-protein N	6.45	7.88	10.05	8.06
Casein N	78.86	74.38	71.77	74.19
Alb. and Glob. N	10.07	9.80	12.43	9.40
Proteose N	4.62	7.80	5.75	8.35

* Albumin and globulin determined together.

The enzyme treated milks show definite protein hydrolysis especially of the casein N fraction. This is more evident in the Trypsin and Enzylac treated milks. The non-casein, non-protein and proteose N fractions of the enzyme treated products have increased in percentage N. The data indicate that the cleavage products from the casein N fraction are determined with the other N fractions and especially with the non-casein N. The more significant increase in non-protein N in this data indicate that a greater degree of hydrolysis has taken place in the protein fractions of this product than in the evaporated milk—(E) table 7.

Average Nitrogen Distribution in Milk. Table 11 shows the average N distribution in five different samples of each of the following products: raw

TABLE 11
Average N distribution in processed milk

	Raw (per cent N)	Past. (per cent N)	Homo. (per cent N)	Skim (per cent N)
Total N	0.5306	0.5356	0.5228	0.5249
Non-casein N	0.1153	0.1106	0.1074	0.1104
Non-protein N	0.0299	0.0298	0.0293	0.0291
Globulin N	0.0176	0.0173	0.0203	0.0184
Casein N	0.4153	0.4249	0.4153	0.4145
Albumin N	0.0511	0.0447	0.0431	0.0410
Proteose N	0.0168	0.0187	0.0146	0.0218
Data above calculated as per cent of total N				
Non-casein N	21.73	20.65	20.54	21.03
Non-protein N	5.64	5.56	5.60	5.54
Globulin N	3.32	3.23	3.88	3.51
Casein N	78.27	79.33	79.44	78.97
Albumin N	9.63	8.35	8.24	7.83
Proteose N	3.17	3.49	2.79	4.15

bulk milk, pasteurized milk 4 per cent fat, 4 per cent milk pasteurized—cooled to 135° F. and homogenized at 2500 lbs. pressure with single stage, and pasteurized skim milk. All the processed products were manufactured from the raw bulk milk.

The data in table 11 show that the processing has produced no significant changes in N distribution.

DISCUSSION

The changes in the N distribution of the homogenized milk (tables 1, 2, 5, and 6) are not significant. There is some evidence in the data recorded in tables 3 and 4 to indicate that there may have been some disturbance of the protein fractions but the evidence is slight and these products have been subjected to pressures that are not used commercially.

The N distribution in evaporated milk (table 7) shows some interesting variations. The most significant changes have occurred as a result of the coagulation of the albumin and globulin—especially albumin. All globulin should be coagulated at 72° C. (3) but complete coagulation, according to the analysis, has not taken place. This is probably due to errors since this fraction contains such a small amount of N. For routine analysis it is more convenient and probably more accurate to determine the albumin and globulin together since the quantitative separation and determination of the small amount of globulin in milk is of questionable accuracy. In order to determine the amount of hydrolysis in the evaporated milk the casein N fraction in (E) is compared with the same fraction in the condensed (C) or the condensed and homogenized milk (D) because the casein N has increased due to coagulated albumin and globulin and this is the only fraction which shows any significant hydrolysis. Small but consistent increases in the amino N of heated milk (4, 5) indicate that some hydrolysis takes place during the heating process. The decrease in the casein N fraction in the evaporated milk (E) is beyond the limits of experimental error. The non-casein N has increased a significant amount, comparing (E) with (C) or (D), while the other N fractions have increased a small amount, so small as a matter of fact that the increase could be attributed to experimental error. However these increases in the N fractions of the evaporated milk with a decrease in the casein N fraction indicate that the proteins in the casein N fraction are in a preliminary stage of breakdown and these cleavage products are distributed and determined with the other N fractions, apparently largely the non-casein N fraction. This trend of thought seems to be supported by the N distribution in the enzyme treated milk (table 10) which shows definite hydrolysis of the milk proteins.

During this study curd tensions were determined on several of the milk products analyzed for N distribution. The curd tension of the evaporated milk (E, table 7) was zero for both the evaporated and the reconstituted

product. The raw milk, pasteurized milk and the homogenized milk (table 1) had curd tensions of 57, 53, and 12 grams respectively. The enzyme treated milk (table 10) which shows the most significant hydrolysis of any of the products had an average curd tension of about 26 grams. If the curd tension is a reliable measure of the consistency of the milk curd it seems reasonable to assume that the greatest alteration in the physical conditions of the milk proteins have taken place in the evaporated milk. There seems to be no direct relationship between N distribution and curd tension because the enzyme treated milks show the most significant hydrolysis and they had the highest curd tension of the three processed products.

One fact should be mentioned concerning the determination of casein N in milk products that have been subjected to heat. The present accepted analytical methods (6) for this determination are subject to error and the magnitude of this error will depend upon the temperature and the length of time the milk has been heated. As milk is heated at increasing temperatures the amount of albumin and globulin coagulated increases and these coagulated proteins are co-precipitated with the casein causing high results for this fraction. The data in table 7 show that the raw milk had a casein content of 0.4152 per cent N which may be considered the normal casein N value for all samples listed in this table. Using this figure (0.4152) as the normal casein N value one finds that a determination of the casein N in the pasteurized milk or in (C), (D) or (E) would result in errors of 0.0110, 0.0753, 0.0789 and 0.0533 per cent nitrogen respectively—if this fraction is to be called casein N. This fraction in all heated milk has been referred to in this report as casein N in order to be consistent and avoid confusion but the term is erroneous when milk is heated sufficiently to cause some

TABLE 12
Nitrogen distribution in sterilized skim milk

	Semimicro analysis	
	Past. skim (per cent N)	Sterilized (per cent N)
Total N	0.5333	0.5270
Non-casein N	0.1103	0.0719
Non-protein N	0.0300	0.0401
Globulin N	0.0197	0.0188
Casein N	0.4230	0.4551
Albumin N	0.0353	0.0000
Proteose N	0.0253	0.0319
Data above calculated as percentage of total N		
Non-casein N	20.68	13.64
Non-protein N	5.63	7.61
Globulin N	3.69	3.57
Casein N	79.32	86.36
Albumin N	6.62	00.00
Proteose N	4.74	6.05

coagulation of the albumin and globulin. It has been stated that the casein N fraction in (E, table 7) shows some hydrolysis but it is difficult to tell what proteins are actually hydrolyzed due to the co-precipitation of albumin and globulin with this N fraction.

The error introduced in the determination of casein N in heated milk may be demonstrated by an analysis of the data in tables 12 and 13. In this experiment pasteurized skim milk was sealed in tin cans and sterilized at 120° C. for 30 minutes. The skim milk and the sterilized skim milk were analyzed for N distribution by semimicro and the Official macro methods (6).

Casein, albumin and residual N² were determined by the Official methods (6) and compared with the same fractions determined by the semimicro methods. The comparison is shown in table 13.

TABLE 13
Comparison of the N distribution in skim milk analyzed by the official and semimicro methods

	Official method		Semimicro method	
	Past. skim (per cent N)	Sterilized (per cent N)	Past. skim (per cent N)	Sterilized (per cent N)
Casein N	0.4050	0.4484	0.4230	0.4551
Albumin N	0.0535	0.0287	0.0353	0.0000
Residual N*	0.0713	0.0791	0.0750	0.0908
(Calculated) Total N	0.5298	0.5562	0.5333	0.5459

* The determination of residual N is not an Official Method. It has been included with these methods for convenience and because the Official Kjeldahl-Gunning-Arnold Method was used to determine the N in this fraction and for all other macro determinations. The filtrate from the albumin N determination was used to determine residual N.

The total N (table 13) is obtained by adding the values for the different N fractions. In the semimicro analysis the values for residual N are obtained by adding the values reported in table 12 for proteose, non-protein and globulin N which would correspond to the residual N determined by the Official method. The residual N reported in the semimicro analysis may be calculated also by subtracting the albumin N from the non-casein N. The increases in the casein N fraction, due to coagulated albumin and globulin, may be observed in the sterilized milk. The casein N fraction determined by semimicro methods has a slightly higher N value than the same fraction determined by the Official methods. This is attributed to several factors which will be discussed later. The errors introduced in the determination of the N fractions of milk by both analytical methods may be observed by the variations in the calculated total N as compared to the actual value 0.5333 per cent N.

A Note Concerning the Separation of the Protein Fractions of Milk by Semimicro Methods. The theories concerning the separations of the protein

² See footnote to table 13.

fractions of milk by semimicro methods have been omitted because they have been comprehensively discussed by Rowland (7, 8).

The following procedure was used to check the semimicro separations of the protein fractions. Samples of reconstituted evaporated milk (some of same milk analyzed in table 7) were weighed into 150 ml. beakers and the milk proteins precipitated as in separations II, III and IV (Procedure). These precipitates were collected on quantitative filter paper and washed with the precipitating agents in the same concentration as they were used in the actual precipitation. The precipitates and paper were transferred to 500-ml. Kjeldahl digestion flasks and the N in these determined by the Official-Kjeldahl-Gunning-Arnold Method (6). The proteins represented by each of these three determinations were compared with the corresponding N fractions obtained by the semimicro N distribution analysis of the evaporated milk. Since only filtrates are used in the semimicro procedure it seemed desirable to analyze the protein precipitates from these separations, thus making it possible to actually check the semimicro separation of the protein fractions of the milk. The heated milk was used for this experiment in order to complicate the separations. The results are shown in table 14.

TABLE 14
Accuracy of protein separations by semimicro methods

Analysis by Semimicro-Kjeldahl		Analysis by Macro-Kjeldahl	
	Per cent N	Precipitate	Per cent N
Total N	0.5342		
Non-casein N	0.0551		
Non-protein N	0.0421		
Globulin N	0.0152	II	0.4843
Casein N	0.4792	III	0.5042
Albumin N	0.0000	IV	0.4844
Proteose N	0.0124		

Precipitate II should contain only casein N, Precipitate III should contain casein, albumin, globulin and proteose-peptone N, while Precipitate IV should contain casein, albumin and globulin N. The N values for these fractions should check with the sum of the corresponding N values reported in the semimicro analysis. The comparisons are as follows:

	Macro-Kjeldahl Precipitates (per cent N)	Semimicro-Kjeldahl Filtrates (per cent N)	Difference (per cent N)
Casein	0.4843 (Ppt. II)	0.4792	0.0051
Casein			
Albumin	0.5042 (Ppt. III)	0.5068	0.0026
Globulin			
Proteose			
Casein			
Albumin	0.4844 (Ppt. IV)	0.4944	0.0100
Globulin			

The checks secured in this separation are satisfactory.

Another experiment of similar nature was conducted in order to compare all results with the present accepted Official Methods (6) for determining the proteins in milk. Pasteurized 4 per cent milk was used for this experiment. Table 15 shows the N distribution in this milk as determined by the Official Methods (in triplicate) and the semimicro methods (in duplicate).

TABLE 15
N distribution in pasteurized milk by official and semimicro methods.
Results reported as per cent N

Official methods				
Total N	Official casein N	Tent. casein N	Albumin N	Residual N
0.5108	0.4008	0.4110	0.0529	0.0626
0.5057	0.3958	0.4021	0.0527	0.0613
0.5131	0.3804	0.4083	0.0518	0.0610
0.5099	0.3923	0.4071	0.0525	0.0616 Ave.
Semimicro methods				
Total N	0.5116	0.5184	0.5150	
Non-casein N	0.1073	0.1080	0.1077	
Non-protein N	0.0309	0.0309	0.0309	
Proteose-peptone plus non-protein N	0.0472	0.0477	0.0475	
Globulin N	0.0134	0.0155	0.0145	
Casein N	0.4043	0.4104	0.4074	
Albumin plus Globulin N	0.0601	0.0603	0.0602	
Albumin N	0.0467	0.0448	0.0458	
Proteose-peptone N	0.0163	0.0168	0.0166	
Residual N (Calculated)	0.0620	0.0619	0.0620	

Casein was determined by the Official and Tentative Methods in order to compare these determinations with the semimicro results for the same fraction. The Tentative Official Method is similar to the semimicro procedure for the determination of this protein and the data indicate that the results secured by each method check very closely. Both of these methods give more consistent checks for casein N than the Official method.

The Tentative Method is not adaptable to routine analyses because unnecessary steps have been introduced into the method which make it too inconvenient for general use. The simplified procedure indicated in separation II (Procedure) will give the same results, by either macro or semimicro methods, as the Tentative method with much less inconvenience to the analyst. Both Rowland (7) and Moir (9) have claimed that the Official Method for casein gives low results because the filtrates from this precipitation have a pH lower than the iso-electric point of casein. The filtrates from the casein precipitations by all three methods were checked and the pH found to be 3.3 for the Official and 4.6 for the Tentative Official and semimicro methods. The last two methods give higher values for casein N than

the Official Method (table 15) and this is attributed to the proper pH of the filtrate (pH 4.6) and the elimination of washing and transferring casein precipitates.

Samples of the same 4 per cent milk used for the previous experiment (table 15) were weighted into 150 ml. beakers and the proteins precipitated as in II, III, and IV (Procedure), or the same procedure was followed as was used to obtain the data shown in table 14. The analysis of the protein precipitates is shown in table 16.

TABLE 16
*Accuracy of semimicro protein separations in 4 per cent milk.
Results in per cent N*

	Ppt. II	Ppt. III	Ppt. IV
	0.3908	0.4758	0.4543
	0.3831	0.4838	0.4531
	0.3908	0.4619	0.4549
Ave.	0.3882	0.4738	0.4541

The average N values for these protein fractions are compared with the corresponding average N values in the semimicro analysis reported in table 15. The results are as follows:

	Macro-Kjeldahl Ppts.	Semimicro-Kjeldahl filtrates	Difference
Casein	0.3882 (Ppt. II)	0.4074	0.0192
Casein Albumin	0.4738 (Ppt. III)	0.4843	0.0105
Globulin Proteose			
Casein Albumin	0.4541 (Ppt. IV)	0.4677	0.0136
Globulin			

The results are very satisfactory when one considers the difficulties involved in an analytical comparison of this nature. These results and those reported in table 14 indicate that the semimicro methods are satisfactory for separating the protein fractions of milk.

Five individual determinations were made on the same sample of pasteurized 4 per cent milk in order to determine the reliability of individual determinations by the semimicro methods. The results are shown in table 17.

Duplicate determinations on the same N fractions by semimicro methods usually give more satisfactory checks than macro methods. The semimicro methods for determining the protein fractions of milk are not above criticism but they are sensitive, well adapted to routine analyses and reliable as indicated by their comparison with the present accepted methods.

TABLE 17
Accuracy of individual determinations by semimicro methods.
Results in per cent N

	Mean	Mean deviation
Total N	0.5187	0.0030
Non-casein N	0.1087	0.0009
Non-protein N	0.0298	0.0009
Proteose-peptone plus non-protein N	0.0493	0.0015
Globulin N	0.0104	0.0032
Casein N	0.4100	0.0026
Albumin and Globulin	0.0598	0.0012
Albumin	0.0488	0.0025
Proteose-peptone	0.0195	0.0024

SUMMARY

1. Semimicro methods have been described and used to study the N distribution in milk. These methods are efficient, well adapted to routine analyses and results compare very favorably with those obtained by the Official Methods.

2. The homogenization of milk at normal and abnormal pressures produced no significant changes in the N distribution.

3. The coagulation of albumin and globulin was the most significant change that occurred in the N distribution of evaporated milk. There is evidence to indicate that some hydrolysis of the proteins takes place in this milk as a result of the processing.

4. Condensing skim milk produced only minor changes in the N distribution of the product.

5. The addition of Steapsin, Trypsin and Enzylac to milk at a temperature of 145° F. for 30 minutes produced definite hydrolysis of the milk proteins.

6. Pasteurization of milk at 145° F. for 30 minutes produced no significant changes in N distribution.

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A STUDY IN CHEESE RIPENING. THE INFLUENCE OF AUTO- LYZED CELLS OF *STREPTOCOCCUS CREMORIS* AND *STREPTOCOCCUS LACTIS* ON THE DEVELOP- MENT OF *LACTOBACILLUS CASEI*

P. ARNE HANSEN

Department of Bacteriology, Georgetown University School of Medicine, Washington, D. C.

There is a gradual change in the bacterial flora of cheese during the process of ripening. In the hard cheeses, which have not been heated to a high temperature, *Streptococcus lactis* and *Streptococcus cremoris* are predominant at first, later being replaced by *Lactobacillus casei* (Orla-Jensen) Holland (*Bacillus casei alpha* v. Freudenreich and Thöni, *Streptobacterium casei* Orla-Jensen), a bacterium which is of importance for the cheese ripening process on account of its proteolytic activity. Many strains of this species show active break-down of casein when inoculated into milk containing calcium carbonate (Orla-Jensen 1919 (1)). No satisfactory explanation for the general occurrence of *L. casei* has so far been given.¹ In the present work a factor for the development of *L. casei* has been studied, namely the influence of the cell content of *Str. lactis* and *Str. cremoris*.

EXPERIMENTAL

In the course of the study the following strains were used:

From the collection of Dr. S. Orla-Jensen, Copenhagen: *Streptococcus cremoris* No. 37, *Str. lactis* No. 7, *Str. lactis* No. 17, *Str. lactis* No. 22, *Beta-coccus cremoris* No. 7, *Streptobacterium casei* No. 7, *Streptobacterium casei* No. 11 and *Thermobacterium helveticum* No. 12.

From the American Type Culture Collection, Washington, D. C.: *Lactobacillus casei* No. 334, *Lactobacillus casei* No. 393 = *Streptobacterium casei*, Orla-Jensen No. 7.

From the collection of Dr. J. M. Sherman, Cornell University, Ithaca, N. Y.: *Streptococcus cremoris* No. C37V.

The streptococci were grown in 4 liters of a peptic casein digest medium, which was made perfectly clear with albumin of egg, $\frac{1}{4}$ per cent being used.

Casein digest adjusted to 0.5 per cent nitrogen.....	1000 ml.
K ₂ HPO ₄	2 g.
MgSO ₄ · 7H ₂ O	1 g.
Glucose	5 g.

adjusted to pH 6.8

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¹ This organism should not be confused with *Lactobacillus helveticus* (Orla-Jensen) Bergey et al. (*Bacillus casei epsilon* v. Freudenreich and Thöni, *Thermobacterium helveticum* Orla-Jensen) which is of the greatest significance for the ripening of Swiss cheese, but, unlike *L. casei*, it is not generally found in all kinds of cheeses.

The cultures were incubated at 20-23° C. until a heavy growth resulted, usually for 2-6 days. The cultures were centrifuged, the supernatant fluid poured off, and the cells were suspended in one liter of physiological saline. The process was repeated, and finally the bacteria were suspended in a small volume of saline, about 40 ml. From then on the bacterial preparations were handled in various ways.

A. *Autolysis of Bacteria*. This was carried out by the following procedure. To the 40 ml. of suspension were added 1.7 g. NaCl and a little toluene, the mixture was well shaken and placed at 49° C. for 5 weeks. The acid formed during the process was neutralized by adding 0.2 N NaOH to a slightly alkaline reaction, the indicator, thymol blue, being used. The suspensions were examined under the microscope, and it was noticed that during this long exposure to 49° C., considerable break-down of the cells occurred, while, *e.g.*, 48 hours treatment seemed quite insufficient to change the appearance of the cells. After evaporation of the toluene on a water bath, the nitrogen content was ascertained and finally the preparation was diluted to a definite nitrogen content and sterilized by autoclaving in a Freudenberg flask.

B. *Disintegration of Bacteria by the Sonic Oscillator* (Chambers and Flossdorf 1936 (2)). The suspension of bacteria in physiological saline, brought to neutrality, and with a little toluene added was subjected to sonic vibration for a period of 10-30 minutes. The toluene was evaporated, nitrogen determined, and the preparation was diluted to a suitable nitrogen content and sterilized by autoclaving.

C. *No special treatment of bacteria other than sterilization*. Sterilization was usually carried out by 15 minutes exposure in the autoclave at 15 lbs. pressure.

In addition to skim milk and casein digest a whey medium was used in one instance, prepared according to a formula of Mr. M. Rogosa, Bureau of Dairy Industry, U. S. Department of Agriculture. Skim milk was heated to 37° C. and sufficient rennet added to form a firm coagulum in about 30 minutes. The curd was cut in rather big pieces, disturbing it as little as possible. The whey was heated to 80° C., cooled and kept in the ice box over night, then centrifuged; finally the supernatant liquid was passed through a Seitz filter EK. The resulting medium was filtered once more through a Seitz filter for sterilization and pipetted into sterile tubes. They were incubated at 30° C. for five days to detect possible contamination.

EXPERIMENT NO. 1

The influence of autolyzed cells of *Streptococcus lactis* (strain 22) on the development of *L. casei* (strains 7 and 11), *Str. lactis* (strain 22), and *Beta-coccus cremoris* (strain 7) was studied. Skim milk to which varying amounts of extracts containing 0.5 per cent nitrogen had been added was

tubed in 10 ml. lots, and sterilized in the autoclave. The tubes were inoculated and incubated for 16 days, the *L. casei* strains at 30° C., *Str. lactis* and *Betacoccus cremoris* at 23° C. At the end of this period the cultures were titrated with NaOH and the results expressed in per cent lactic acid formed.

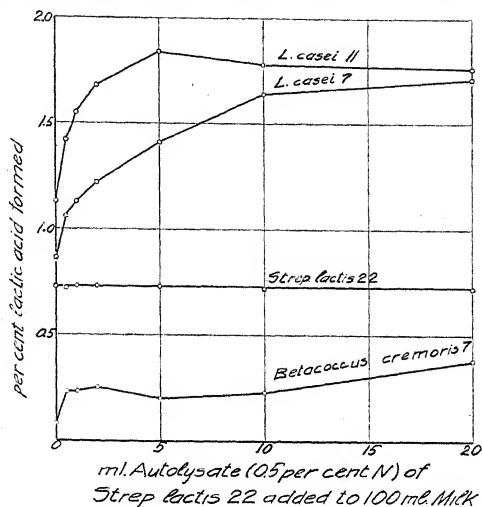


FIG. 1. Influence of autolyzed cells of *Str. lactis* 22 on acid formation by *L. casei* 7 and 11, *Str. lactis* 22, and *Betacoccus cremoris* 7.

The data in figure 1 show a considerable increase in the endpoint of fermentation for the *L. casei* strains and for *Betacoccus cremoris* 7; but *Str. lactis* 22 was not influenced by extracts of its own cells.

The activity was not limited to extracts of this strain as the following results show.

EXPERIMENT NO. 2

The experimental procedure was in the main the same as in No. 1 with the exception that the media in which the various *Strs.* were grown contained 1 per cent glucose and that the preparations of autolyzed cells were made up to 1 per cent nitrogen. Autolysates of *Str. lactis* (strains 7 and 17) and of *Str. cremoris* (strain 37) were added to milk as in the previous experiment. The organisms grown in the extracts were *L. casei* (strains 7 and 11) at 30° C. and *L. helveticus* (strain 12) at 37° C. *L. casei* was encouraged, as will be noted from figure 2, while *L. helveticus* seems not to be influenced favorably, but rather inhibited.

EXPERIMENT NO. 3

A suspension of *Str. cremoris* (C37V) was prepared and divided into two parts. One was left without special treatment and the other was shaken by sonic vibration for 10 minutes, the object being to ascertain whether the

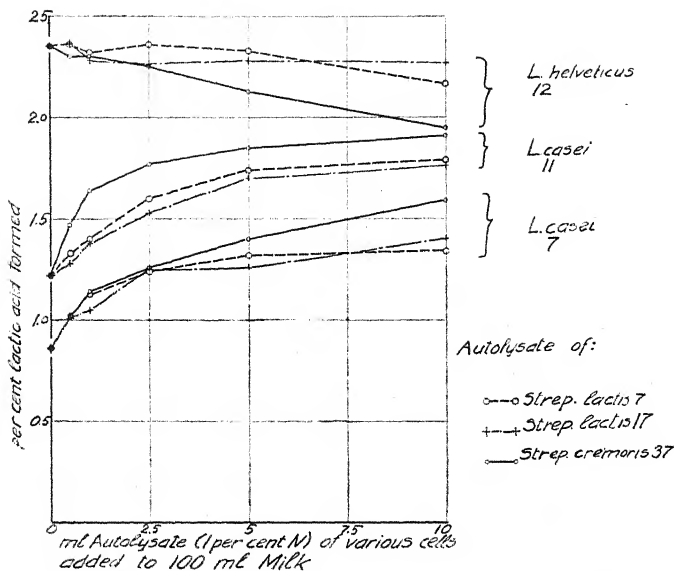


FIG. 2. Influence of autolyzed cells of *Str. lactis* 7 and 17 and *Str. cremoris* 37 on *L. helveticus* 12, *L. casei* 7 and 11.

breaking-up of the cells actually has any significance or whether an untreated plain cell suspension would not be just as active. One ml. of different dilutions of the suspensions was added to 9 ml. milk or whey, bringing the final volume to 10 ml. The resulting culture media tubes were inoculated and then incubated at 30° C.

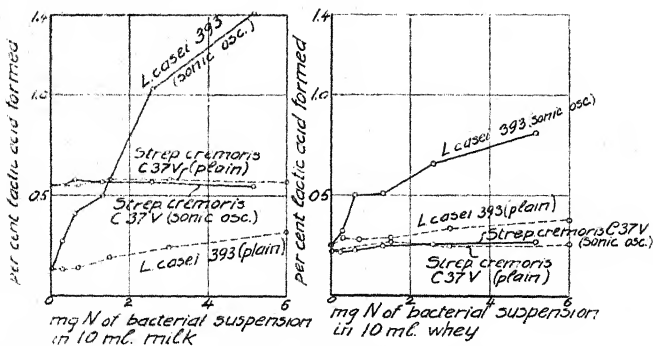


FIG. 3. Influence of cell suspensions of *Str. cremoris* C37V on acid formation of *L. casei* 393 and *Str. cremoris* C37V in milk and whey.

Str. cremoris extracts have no influence on the development of a culture of the same strain; but the *L. casei* is very favorably acted upon, and the disintegrated cells of *Str. cremoris* are far more potent than the whole cells (see fig. 3).

TABLE 1

Endpoint of fermentation produced in milk to which cells and filtrates from cells of *Streptococcus cremoris* were added

Composition of media	<i>L. casei</i> 334		<i>L. casei</i> 393	
	Per cent lactic acid formed	Days to curdle	Per cent lactic acid formed	Days to curdle
S.1 ml. skim milk + 0.9 ml. physiol. saline	1.38	3	0.19	Did not
S.1 ml. skim milk + 0.9 ml. suspension, sonic vibrated (A)	1.49	3	0.65	3
S.1 ml. skim milk + 0.9 ml. plain suspension (B)	1.43	3	0.28	Did not
S.1 ml. skim milk + 0.9 ml. Seitz filtrate of A	1.52	3	0.65	3
S.1 ml. skim milk + 0.9 ml. Seitz filtrate of B	1.40	3	0.25	Did not

A = sonic vibrated suspension, 3.19 mg. N/ml.

B = plain vibrated suspension, 3.17 mg. N/ml.

TABLE 2

Endpoint of fermentation and growth produced in casein digest to which filtrates from cells of *Streptococcus cremoris* were added

Composition of media	<i>Streptococcus cremoris</i> C37V			<i>Lactobacillus casei</i> 334			<i>Lactobacillus casei</i> 393		
	Per cent lactic acid formed	Turbidity		Per cent lactic acid formed	Turbidity		Per cent lactic acid formed	Turbidity	
		E	$\frac{E}{E_{control}}$		E	$\frac{E}{E_{control}}$		E	$\frac{E}{E_{control}}$
Control. 4 ml. casein digest, double strength, with 80 mg. glucose + 4 ml. saline	0.38	0.40	1.0	0.69	0.26	1.0	0.31	0.20	1.0
+ 1 ml. casein digest, double strength, with 80 mg. glucose + 1.2 ml. filtrate from A + 2.8 ml. saline	0.52	0.48	1.2	0.56	0.51	2.5
+ 1 ml. casein digest, double strength, with 80 mg. glucose + 2.4 ml. filtrate from A + 1.6 ml. saline	0.53	0.49	1.2	0.91	0.71	2.7	0.80	0.61	3.0
+ 1 ml. casein digest, double strength, with 80 mg. glucose + 2.4 ml. filtrate from B + 1.6 ml. saline	0.48	0.44	1.1	0.92	0.61	2.3	0.44	0.33	1.65

A = sonic vibrated suspension.

B = plain suspension.

E = Extinction = $\log_{10} \frac{100}{\% \text{ transmission}}$

EXPERIMENT NO. 4

The experiments so far described were concerned with the endpoint of fermentation; but the crop of bacteria produced cannot be predicted from such data. Bacterial counts are difficult to evaluate in case of chain-forming rods, since an unbroken chain or a very long rod may produce one colony only as does a single short rod, when the plate method is used. Turbidity measurements were used in the present work; they seem to give satisfactory results, provided the apparatus is operated with a range of about 60–25 per cent transmission or an extinction of 0.22–0.60, and provided a sufficient number of control tubes are being run simultaneously with the inoculated tubes. The apparatus used was an Aminco Photometer, Type F, equipped with filter 58. Obviously a clear medium must be used; thus milk had to be abandoned and a casein digest containing one per cent glucose was used instead. The bacterial extract was prepared by shaking a suspension of cells for 30 minutes on the sonic oscillator, strain C37V of *Str. cremoris* being used. Besides studying the growth as determined by increase in turbidity the object of the present experiment was to determine whether the active principle would pass a filter. After inoculation the cultures were incubated at 30° C. for 30 days. The turbidity was always measured in relation to the corresponding noninoculated medium, but containing the various additions. Simultaneously another experiment using skim milk as a basic medium was run for the sake of comparison.

DISCUSSION

There is no proportionality between the amount of growth and the endpoint of fermentation. Yet it may be stated that for each single strain an increase in endpoint always corresponds to an increase in growth through the range investigated.

Figures 1 and 2 indicate definitely an increased development of all *L. casei* strains and of *Betacoccus cremoris*. The latter species is found in starters for butter-making together with *Str. lactis* and *Str. cremoris*. Although it develops scantily in pure culture in skim milk, it is found in starters, which have been transferred for years. The favorable influence of the cell content of the streptococci on the development of *Betacoccus cremoris* may be a significant factor. *L. helveticus* was not encouraged in the experiments here described; but it must be remembered that skim milk is already a very good culture medium for *L. helveticus* and that a different result may have been obtained if another basic medium had been used for the study. The decrease in endpoint with very large additions of extracts was undoubtedly caused by the fact that this organism is very sensitive to sodium chloride. Thus a somewhat distorted picture resulted when large amounts of autolyzed cells were added. (See figs. 1 and 2.) In the most striking case the final medium with 10 ml. extract of *Str. cremoris* 37 added per 100

ml. milk, contained 0.49 per cent sodium chloride. The two other extracts, of *Str. lactis* 7 and 17, had a smaller sodium chloride content per one per cent nitrogen, because the crops of these species were larger than those of *Str. cremoris* 37.

Figure 3 shows that even the suspension of untreated cells of *Str. cremoris* C37V were somewhat active; however the sonic vibrated cell suspension was very much more active towards *L. casei*. Two weeks intervened between the preparation of the sterile cell suspension and the starting of experiments No. 3 and 4, and some of the cell content may have passed into the surrounding medium during the storage. The data presented show the importance of using rigorous methods for liberating the cell content, at least when streptococci are studied.

The active principle seems to be filtrable as indicated by tables 1 and 2. The much greater activity of sonic vibrated suspensions as compared with control suspensions has also been demonstrated in this experiment. The strain *L. casei* 334 is less sensitive to additions than the other *L. casei* strains when acid formed is used as an indicator. The data in table 2 show that also with a casein digest medium stimulation of the *L. casei* strains measured either by turbidity or by acid formation can be demonstrated. Under the conditions of these experiments the growth was increased 2.7 times for *L. casei* 334 and 3 times for *L. casei* 393.

In this work the nitrogen content of the various extracts was determined with the object of roughly standardizing the strength of the active preparations. However the activity varied considerably and the nitrogen content is only a rough guide to the potency of the extracts. The endpoint of fermentation may, of course, vary a little with different lots of milk. Although this difference is usually insignificant the same lot of milk was always used throughout each single series of experiment.

CONCLUSIONS

1. Extracts of *Streptococcus cremoris* and *Streptococcus lactis* stimulate the development of *Lactobacillus casei* and *Betacoccus cremoris* (*Streptococcus citrovorus*). Both the endpoint of fermentation and the final bacterial crop are increased. However, no or only faint stimulation is observed when *Streptococcus lactis* and *Streptococcus cremoris* are grown in media enriched by their own cell content.

2. Preparations of autolyzed cells or sonic vibrated cells are much more potent than non-treated control suspensions of killed cells.

3. The active principle is filtrable through a Seitz filter EK.

4. The stimulation of *Lactobacillus casei* by *Streptococcus cremoris* and *Streptococcus lactis* may play an important role in the ripening of cheese, where these species are found at first, then die and disintegrate and are followed by *L. casei*. Furthermore the phenomenon may be of importance in

butter and cheese cultures where the two streptococci are found mixed with aromabacteria.

5. The sonic oscillator is a valuable tool for the study of associated growth of microorganisms.

ACKNOWLEDGMENT

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THE EFFECT OF VITAMIN A AND CERTAIN MEMBERS OF THE B-COMPLEX UPON CALF SCOURS¹

PAUL H. PHILLIPS, NORMAN S. LUNDQUIST AND PAUL D. BOYER

*From the Departments of Biochemistry and Dairy Husbandry,
University of Wisconsin, Madison, Wisconsin*

The causal factor or factors involved in "scours" in the early life of the calf have been largely considered to be bacterial in nature. There is little doubt that infectious agents are seriously involved and particularly so in the case of "white scours." That nutrition or the nutritive state of the calf is likewise of some importance in the course of such diseases has been taken for granted but little questioned. To our knowledge no specific nutritional researches have been made which would definitely indicate the role of nutrition as it relates to scours. Krauss and coworkers (4) have shown that the amount of carotene and vitamin A in the liver of the new-born calf is extremely low. It has long been known that colostrum milk is rich in vitamin A. Further, the function of vitamin A has always been associated with disease resistance.

In view of these facts and the rather common appearance of diarrhea among calves of certain herds under our observation, a field study was made to determine the effect of vitamin A upon the control of scours in the new-born calf.

Several herds which had been under observation for reproductive troubles first caused us to turn our attention to their calf crop. Generally speaking the cows in these troublesome herds which were causing concern were low in vitamin C and responded to both vitamin C and A treatment (8). Calves from these herds had shown the following history: The calves were apparently normal at birth. They were able to nurse but usually went "off feed" between the second and seventh days with early evidence of an effusive watery diarrhea, or scours. Frequently the feces bordered on the "white" type of scours and not infrequently there was some evidence of hemorrhage. Often there was excessive lacrimation although this was not a constant symptom. The course of the disease usually went into pneumonia in its final stages and death from the latter cause. Before treatment was instituted the disease proved to be fatal in 50 to 75 per cent of the cases and those which withstood the attack were greatly retarded in their development. Two herds, one a Holstein and the other a Jersey, were available for study together with a few isolated cases. In both herds the amount of milk fed was rigidly reduced with the onset of the diarrhea. In the case of the Jersey

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calves they were given regularly only 3 lbs. and the Holstein calves about 5 lbs. milk each per day. The owner of the Holstein herd had appealed to the State Sanitation Laboratory for aid. Upon their recommendation the pregnant cows were injected with a bacterin preparation before the calves were dropped and the new-born calves were immediately treated with the serum at birth. This routine had been in practice without favorable results for some two months prior to our vitamin A and C analyses and subsequent vitamin therapy. Carotene and vitamin A analyses were made with the Evelyn colorimeter using Kimble's method (3). The constant for the conversion of the L values into gamma of vitamin A was obtained from measurements with pure vitamin A.² Vitamin C analyses were made according to the Mindlin and Butler method (6).

Shark liver oil³ containing 15,000 I.U. of vitamin A per gram was used as the source of vitamin A. It was fed in 5 cc. gelatin capsules at the rate of 10 cc. per week. In most cases the calves were fed for 2 or 3 weeks depending upon their condition.

RESULTS

The Holstein calves were treated with shark liver oil. In general there was an immediate response as shown by the improvement in appetite and general appearance of the calves. Diarrhea was only partially controlled. There was some temporary improvement in all the treated calves. However, only about half of the calves recovered sufficiently to show no effects of the disease. Those which did not recover were subsequently treated with certain members of the B complex as described below.

The second herd to be treated was a Jersey herd where the mortality was extremely high. Calf scour remedies were tried without success. Since we had not attained 100 per cent response in the Holstein calves to vitamin A administration it was decided to consider the administration of other vitamins as well. Studies on the pathology of nutritional diseases had shown in our laboratory (9) that (1) a nicotinic acid deficiency caused diarrhea in the dog, (2) that a pantothenic acid deficiency caused marked congestion of the entire intestinal tract and diarrhea in both the rat and dog, often accompanied by evidence of hemorrhage, and (3) that vitamin A deficiency likewise caused diarrhea. On the basis of these studies with other species and at the suggestion of Dr. D. F. Green of Merck and Co., certain members of the vitamin B complex³ were obtained and fed. From analyses for the various members of the vitamin B complex in milk it was estimated from the known requirement of other species that the calf (100 lbs. live weight) would require approximately 10 lbs. of milk daily to supply his vitamin B com-

² Determined by Dr. F. W. Quackenbush and H. L. Gottlieb.

³ We are indebted to Bioproducts Inc., Astoria, Oregon, for the generous supply of shark liver oil and to Merck and Co., Rahway, New Jersey, for their generous supply of the crystalline vitamin B complex used in these experiments.

plex requirements. The first experiments on 6 Jersey calves indicated that nicotinic and pantothenic acids together with shark liver oil completely controlled the scours in these calves. Since the calves were on a reduced milk intake and the management desired to continue them thus, other members of the B complex were added. Therefore the results with these calves were obtained on nicotinic and pantothenic acid for the first two weeks and the other B vitamins added for an additional 2 weeks. These vitamins were given in weighed quantities in capsules once per week at the rate of:

Vitamin B ₁	1 mg./day
Riboflavin	1 " "
Pantothenic acid	5 " "
Choline	5 " "
Nicotinic acid	10 " "

The results from these studies are shown in table 1. It is apparent that the vitamin A content of the blood plasma of these calves was low at the beginning of these experiments. There was improvement in their general health and a reduction in the number of cases of scours as the result of feeding small amounts of shark liver oil. The vitamin A content of the blood rose sharply as the result of vitamin A feeding and continued throughout the period that the calves were under observation. The rise in blood plasma A was accompanied by a rise in the ascorbic acid in those calves which were deficient in vitamin C at the beginning of the experiments.

The vitamin B complex members which were given had an immediate and dramatic effect upon the calves' diarrhea. With the addition of the B complex in the presence of adequate vitamin A the feces returned to a normal consistency within 12 to 24 hours. Mortality in these calves was reduced to zero. Calves subsequently dropped in these herds were treated either with colostrum or shark liver oil and a week or two week's supply of the B complex given shortly after birth. No symptoms of scours developed. The preliminary data with the Jersey calves indicated that nicotinic and pantothenic acids were the members of the B complex which were the effective ones.

Some 8 to 12 cases of mild to severe calf "scours" in the University herd have since been treated with equal success. One case in particular is worthy of mention. A bull calf placed on a ration of mineralized whole milk containing Fe, Cu, Mn and Mg for a year developed stiffness. With the onset of stiffness a watery diarrhea began which continued for 4 or 5 weeks. This became increasingly worse. B complex capsules were administered at twice the rate given to the young calves. The diarrhea completely disappeared within 6 days with a marked improvement by the end of the third day.

These studies raised the question as to the blood plasma content of vitamin A and C of the new-born calf. As many newly dropped calves as could

TABLE 1
Effect of vitamin A upon the progress of calf scours and certain blood constituents

Calf	Vitamin A (gamma/ec.)				Carotene (gamma/ec.)				Ascorbic acid (mgs./100 cc.)			
	1/8/41	Scours	2/12/41	Scours	4/24/41	Scours	1/8/41	2/12/41	4/24/41	1/8/41	2/12/41	4/24/41
Holstein*												
76	.09	Severe	.21	Nil	.14	Nil	.171331
47	.09	"	.18	Mild	.23	"	.26	.28	.66	.18	.29	.61
44	.08	"	.17	"	.21	"	.17	.23	.32	.20	.32	.26
45	.10	"	Nil	.16	"	.4176	.3322
9	"	.19	"	.16	"49	.7249
39	"	.14	Mild	.21	"14	.1526	.52
	3/14/41		3/27/41		4/24/41		3/14/41	3/27/41	4/24/41	3/14/41	3/27/41	4/24/41
Jersey†												
65	.10	Severe	.26	Nil	.09	Nil	.31	.37	.12	Trace	.40	.28
85	.08	"	.28	"	.14	"	.19	.29	.04	"	.26	.32
93	.07	"	.20	"	.14	"	.18	.13	.09	.42†	.21	.37
95	.10	"	"	"	.87	Trace
84	.19	Very slight	.19	"	.13	"04	.05	"	.68	.23
92	.16	"	"	.18	"	.18	.13	.09	"34

* Shark liver oil 1/8/41 → 2/12/41, shark liver oil + B-complex 3/27/41.

† Shark liver oil.

‡ New-born calf.

be obtained before suckling were bled and the vitamin A, C and carotene content determined. The results are summarized in table 2. It is evident

TABLE 2

Summary data on the average vitamin A, C and carotene values of the new-born calf

Age	No. calves	New-born	No. calves	1 day	No. calves	2 days	No. calves	1 week	No. calves	3 weeks	No. calves	6 weeks
Vitamin C (mgs./100 cc.) ...	15	.53	4	.22	4	.29	8	.26	4	.35	5	.30
" A (γ /cc.)	16	.04	4	.14	3	.19	9	.18	5	.16	5	.18
Carotene (γ /cc.)	18	.05	4	.15	4	.20	7	.21	3	.13	5	.16

that the blood ascorbic acid is extraordinarily high in the new-born calf. It is also evident that the vitamin A content of the blood was far below normal, and in the region where a vitamin A deficiency develops (2). By the end of 24 hours the vitamin C dropped about 50 per cent and the vitamin A values rose some 3 to 4 times its original value, thus both moved toward and into the normal range. If the calves were segregated into groups depending upon the ration of their dams, there was some evidence which showed that calves from cows on average winter rations carried 0.04 gamma of vitamin A per cc. as compared to 0.08 gamma per cc. for the calves from dams fed extra quality hay and silage with their winter ration. The data further indicate that the new-born calf was amply fortified with ascorbic acid and that it ingested and obtained large amounts of vitamin A from colostrum milk. Thereafter the blood plasma values for these constituents remained normal under average conditions.

DISCUSSION

These observations again emphasize that the liberal use of colostrum in feeding calves is good management since it supplies the early requirement for vitamin A in the calf.

These studies show that the growing calf benefits from the administration of certain members of the vitamin B complex. This may account for the results obtained by Newman and Savage (7), who showed that brewers yeast in calf starters promoted greater growth in the young calf. Bechdel and associates (1), Wegner *et al.* (10) and McElroy and Goss (5) have shown that the ruminating bovine obtains large amounts of the known members of the vitamin B complex through the activity of bacteria in the rumen. In the calf fed milk only, the rumen does not seem to provide optimum conditions for synthesis of the B complex or else the requirements of the growing calf exceed the ability of the bacteria to synthesize the necessary amounts. It would seem likely that the rumen in the early life of the calf is not suffi-

ciently developed for the establishment of an adequate bacterial flora for the synthesis of the B complex.

On the basis of this work further studies on the nutrition of the new-born calf are being made.

CONCLUSIONS

These studies indicate that the calf diarrhea encountered was largely nutritional in origin. The administration of high vitamin A potency shark liver oil and certain members of the vitamin B complex eliminated the diarrhea and the resulting mortality from pneumonia. Preliminary evidence would suggest that nicotinic and pantothenic acids may be the factors of the B complex which were lacking.

New-born calves were found to be amply fortified with ascorbic acid but they were uniformly deficient in vitamin A. The ingestion of colostrum milk rich in vitamin A quickly brought about normal blood plasma levels. The ration of the dam only slightly influenced the amount of vitamin A found in the blood plasma of the new-born calf. Winter rations tend to reduce it while rations with ample carotene or fortified with vitamin A tend to raise it. Low ascorbic acid values in the blood plasma were increased by feeding shark liver oil rich in vitamin A.

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ESTIMATION OF INITIAL LIVE WEIGHT AT EACH LACTATION OF DAIRY COWS¹

W. L. GAINES

Illinois Agricultural Experiment Station, Urbana

H. P. DAVIS AND R. F. MORGAN

Nebraska Agricultural Experiment Station, Lincoln

For the purpose of this paper it is accepted that an estimate of live weight of the cow should be an integral part of each lactation record in all milk recording work. With reference to D.H.I.A. work, and other systems based on periodic visits of a tester, there are advantages to the routine in which the tester estimates the live weight of each cow that has calved since the last test of the particular herd. In connection with the subsequent lactation record of the cow, this estimate is designated as initial live weight. Such a record of initial live weight will serve the most important needs with a minimum of effort for the tester—a necessary consideration in view of the multitude of detail he has to look after.

The primary object of this paper is to develop and present a method of estimating initial live weight by use of a linear measurement of chest girth. Ragsdale, Brody, Davis, Morgan (1, 2, 3) have heretofore published tables for estimating live weight in pounds (W) of female dairy cattle from chest girth in inches (G) based on application of the equation, $W = aG^b$, to a total of 15,610 pairs of girth-weight measurements, in a large number of subdivisions by age and breed of animal. The observations were made at the Missouri and Nebraska Stations.

In the present paper, the Nebraska observations are reexamined from the special point of view of estimating initial live weight (initial weight in distinction to final weight, average weight, miscellaneous weight, undefined weight). Ample evidence has accumulated showing that initial live weight is a very important datum in the biological consideration of milk production problems. This paper proceeds from the foundation of the previous work cited to develop the detail of a plan of estimating initial live weight of each cow at each lactation, adapted to practical use in D.H.I.A. and other milk recording work.

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¹ This paper is the result of a cooperative arrangement between the Nebraska and Illinois Stations, whereby the chest-girth and live-weight measurements made at the Nebraska Station over the past 19 years have been utilized at the Illinois Station to derive a formula for the purpose of estimating live weight from chest girth at one particular stage of lactation, namely, within the first 31 days after calving. Printed with the approval of the Director of the Nebraska Agricultural Experiment Station as Journal Series 292; and with the approval of the Director of the Illinois Agricultural Experiment Station.

EXPERIMENTAL OBSERVATIONS AND ANALYTICAL PROCEDURE

The observations used in the present paper were made at the Nebraska Station, from 1922 through 1940. Measurements of chest girth and live weight (and many other items not here utilized) were made at approximately the middle of each calendar month for the 19 years indicated on cows in the Nebraska Station herd. At the Illinois Station the girth-weight data have been transcribed to punched cards, one card for each lactation, starting with the first observation after calving in each lactation and continuing month by month thereafter as long as the succession was not interrupted up to and including the tenth month after calving. As the data are used this is the limit of capacity for a single card. As indicated, the primary concern of this paper is with the first observation after calving, to estimate initial live weight.

From the tables previously cited, it is known that both age and breed affect the girth-weight relationship. For example, a mature Holstein weighs about 200 pounds more than a 2-year-old Jersey of the same chest girth. Scrutiny of the tables and other considerations suggest age distinctions, sufficient for practical purposes, as follows: Cows under 3 years of age (< 3 yrs.); cows 3 or 4 years of age (3, 4 yrs.); cows 5 years of age or older (5, + yrs.). Age is reckoned as of date of measurement, that is, the age increases with advance in lactation are recognized.

Study of the previous tables cited suggested the possibility of combining the many equations in a single one, $W = a(G + g)^b$, in which g is an age-breed girth modifier, and still retain the accuracy of weight estimate associated with the age-breed distinctions. Indeed, if there were a single, known, true value for b , the single new equation using this true value of b , would be better than the many old equations, for general application. That is, while any one of the old equations is the best fit to the particular set of girth-weight observations from which it is derived it is not the best general equation for other similar observations if its b differs from the true value of b assumed to exist. It might be assumed from geometrical considerations (volumes of similar solids vary as the third power of corresponding linear dimensions) that the true value of b is 3. The old equations show, however, that the exponent 3 does not hold within age-breed groups. It may be doubted, therefore, that there is any single true value of b , valid for all age-breed groups. Nevertheless, it may be possible to select a single compromise value for b , applying satisfactorily to all age-breed groups, for the practical purpose of estimating initial live weight. If such a satisfactory value of b can be selected it is a simple matter to find the rest of the new equation, numerically. Our first step is to find the value of b from the observations of girth and weight for each of the age-breed groups for the first month of lactation.

Three formulas which may be used to compute the value of b in the equation, $W = aG^b$, are as follows:

1. $b_1 = \frac{n [S (\log G) (\log W)] - (S \log G) (S' \log W)}{n S (\log G)^2 - (S \log G)^2}$
2. $b_2 = \frac{SG^{2b} [S (G^{2b}) (\log G) (\log W)] - [S (G^{2b}) (\log G)] [S (G^{2b}) (\log W)]}{SG^{2b} [S (G^{2b}) (\log G)^2] - [S (G^{2b}) (\log G)]^2}$
3. $b_3 = \frac{[nSGW - (SW)(SG)]/SW}{[nSG^2 - (SG)^2]/SG}$

The first formula is the usual way of computing b . It minimizes $S (\log W_o - \log W_c)^2$ or $S [\log (W_o/W_c)]^2$, where W_o is observed W , and W_c is W computed by the fitted equation. It is the correct least-squares procedure, if we speak in terms of logarithms of the observations and not the observations themselves, and if G is considered to be without error.

The second formula, from Deming (4, page 147 and corrected page 143) minimizes $S (W_o - W_c)^2$. It is the correct least-squares procedure, if we speak in terms of the observations themselves (not their logarithms) and if G is considered to be without error. Since b itself is involved in this formula a necessary first step is to choose a value for b which is not too far from the b which is finally found by formula 2. This first approximation of b may be gotten by any method that gives a fair approximation. To hold G^{2b} within bounds any constant multiplier may be introduced, such as .00001.

The third formula is an approximation derived as follows. A straight line, $W = a' + b'G$, is fitted to the observations by least squares. The slope of this line is b' . The slope of $W = aG^b$ is baG^{b-1} and this is taken at the mean girth (\bar{G}) to be the same as that of the straight line. Then $ba\bar{G}^{b-1} = b'$, or multiplying through by \bar{G} , $ba\bar{G}^b = b'\bar{G}$. Next it is taken that the fitted power equation gives a value of W at the mean girth (\bar{G}) which is the same as the observed mean weight (\bar{W}), whence $a\bar{G}^b = \bar{W}$. Substituting and transposing, $b = b'(\bar{G}/\bar{W})$. In formula 3, SG/SW is substituted for \bar{G}/\bar{W} . The remainder of the formula, the part in square brackets, is simply a least-squares expression for b' . The formula is thus set up for efficiency in machine computation (including the electrically controlled punched card machines) dealing directly with the observations, and avoiding logarithms.

It is presumed that formula 3 may be used safely where the amount of curvature involved in $W = aG^b$ is not too great. Among the groups with which we are here dealing, the Jersey < 3 years records may be expected to involve the greatest amount of curvature. As a test of the applicability of formula 3, all 3 formulas have been applied to the 68 pairs of girth-weight measurements of this group, with the results: $b_1 = 1.799$; $b_2 = 1.795$; $b_3 = 1.789$.

If we are concerned with a least-squares solution of b in terms of the observations themselves (not their logarithms) we find, in this particular

case, $b = 1.795$. Accordingly, formula 1 is to be regarded as an approximation which gives $b = 1.799$, an error of 4 in the third decimal; while formula 3, frankly an approximation at the outset, gives $b = 1.789$, an error of 6 in the third decimal.²

TABLE 1

Value of b in the equation, $W = aGb^b$ (heavy face), and number of pairs of girth-weight measurements (light face) according to breed, age, and month of lactation

Month	Ayrshire			Guernsey			Holstein			Jersey		
	< 3 yrs.	3, 4 yrs.	5, + yrs.	< 3 yrs.	3, 4 yrs.	5, + yrs.	< 3 yrs.	3, 4 yrs.	5, + yrs.	< 3 yrs.	3, 4 yrs.	5, + yrs.
1	2.07	2.23	1.02	2.01	1.99	2.22	1.61	1.82	1.98	1.79	1.71	1.87
	61	53	11	34	26	2	112	126	79	68	71	22
2	2.23	1.80	1.50	2.05	1.84	minus	1.82	1.89	1.86	1.96	2.00	2.26
	55	50	9	32	26	2	104	125	76	65	67	20
3	2.08	2.09	1.61	2.10	2.11	minus	1.91	2.23	1.89	1.84	1.89	2.55
	52	47	9	30	26	2	95	126	76	61	63	21
4	2.43	2.19	2.25	2.07	2.28	1.91	2.31	1.69	2.14	1.99	2.36
	51	43	9	27	25	1	77	132	77	58	58	23
5	2.17	2.36	2.40	2.21	1.78	0.31	1.93	2.17	1.55	2.33	1.90	2.20
	47	42	8	24	23	2	61	140	74	55	59	22
6	2.51	2.33	2.93	2.26	2.23	1.38	2.08	2.41	1.85	1.89	1.99	2.34
	44	44	7	23	21	4	54	137	74	50	60	17
7	1.86	2.16	2.91	2.65	2.24	1.83	1.93	2.13	1.75	1.86	1.81	1.80
	41	44	7	21	18	6	47	136	70	42	63	18
8	1.75	2.49	2.75	1.74	2.35	2.64	2.13	2.12	1.83	2.02	1.86	2.03
	37	41	9	18	19	7	37	133	75	40	59	19
9	1.92	2.16	2.66	1.63	1.83	2.77	2.22	2.02	1.99	2.19	1.69	1.58
	29	41	12	13	24	4	27	133	73	35	56	25
10	2.32	2.28	2.83	1.15	0.92	2.23	2.08	2.17	1.75	2.21	1.64	1.85
	22	45	10	10	26	4	17	131	76	23	63	19
1, 2	2.14	2.01	1.22	2.03	1.89	1.58	1.71	1.86	1.93	1.85	1.83	2.05
	116	103	20	66	52	4	216	251	155	133	138	42
3, 4	2.25	2.14	1.97	2.07	2.20	minus	1.91	2.27	1.78	1.98	1.93	2.45
	103	90	18	57	51	3	172	258	153	119	121	44
5, 6	2.30	2.35	2.65	2.24	1.92	1.27	2.01	2.28	1.70	2.05	1.94	2.25
	91	86	15	47	44	6	115	277	148	105	119	39
7, 8	1.82	2.31	2.82	2.22	2.29	2.10	2.02	2.12	1.79	1.94	1.83	1.90
	78	85	16	39	37	13	84	269	145	82	122	37
9, 10	2.11	2.21	2.76	1.39	1.27	2.46	2.15	2.10	1.86	2.20	1.67	1.67
	51	86	22	23	50	8	44	264	149	58	119	44
All 10	2.19	2.24	2.36	2.11	1.91	1.95	1.89	2.13	1.83	2.04	1.83	2.08
	439	450	91	232	234	34	631	1319	750	497	619	206
All 10 (all ages)		2.32			2.23			2.10			2.08	
		980			500			2700			1322	

² In the present case all 3 formulas give essentially the same result, presumably because the amount of curvature involved in the power equation is not great. If, however, all ages from birth to maturity (e.g., 2, fig. 5) with the associated wide range in size of animal are thrown together, formulas 1 and 2 may give (and in the instance cited do give) very different values of b . (Incidentally, a similar situation may develop in expressing "basal metabolism" as a power function of live weight when animals of widely varying live weights, from "mouse to elephant," are thrown together.) Relatively speaking, formula 1 is more sensitive to deviations at low values of G and formula 2 is more sensitive to deviations at high values of G .

For present purposes it seems that the use of formula 3 is fully warranted, to avoid the extra labor involved in using formulas 1 or 2, applied to the individual pairs of girth-weight measurements. Formula 3 has been used to compute the value of b for each of the several age-breed groups by stage of lactation and the values of b thus derived are given in table 1, together with the respective frequencies. In figure 1 the b values are plotted against stage of lactation to bring out any trends in b with advance in lactation.

INITIAL LIVE WEIGHT EQUATION

The values of b in figure 1 show more or less distinct trends with advance in lactation. For purposes of estimating initial live weight it appears desirable, therefore, to deal with observations at an early stage of lactation, rather than to include all 10 months. On the other hand, the greater the number of observations the more reliable the resulting equation, other things being equal. A compromise is made by using the first two months of lactation in deriving an initial weight equation.

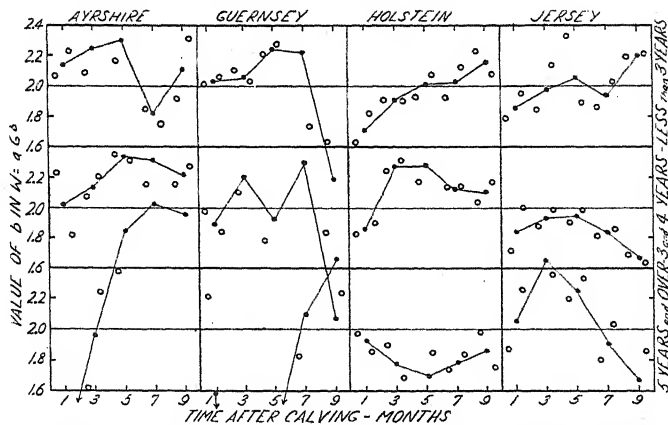


FIG. 1. Values of b in the equation $W = aG^b$ by stage of lactation for each of the age-breed groups. The solid circles connected by straight lines represent two-month periods. The open circles represent one-month periods, but do not include values which fall outside the limits 1.6-2.4.

From the data of table 1 and figure 1, it is concluded that the compromise, $b = 1.85$, is fairly applicable to all groups, for the particular purpose of estimating initial live weight. From table 2 the Jersey < 3 years group has an average weight of 819 pounds and average girth of 67.1 inches and a is computed as $a = 819 / (67.1)^{1.85} = .342$. As a general equation, we now have $W = .342 (G + g)^{1.85}$ where $g = 0$ for the Jersey < 3 years group. The girth-weight table of figure 2 is derived from this equation.

For the Jersey 3,4 years group the average weight is 922 and the average girth is 69.6. The $(G + g)$ value corresponding to 922 in figure 2 is 71.5 and

TABLE 2
Average chest girth, \bar{G} , in inches and average live weight, \bar{W} , in pounds

Breed	Age	Month or months of lactation					
		First one		First two		First ten	
	<i>years</i>	\bar{G}	\bar{W}	\bar{G}	\bar{W}	\bar{G}	\bar{W}
Ayrshire	< 3	71.2	976	71.2	970	71.6	1003
do	3, 4	74.0	1104	73.7	1092	74.4	1121
do	5, +	76.1	1135	76.2	1132	76.3	1164
Guernsey	< 3	70.0	928	70.1	931	71.0	968
do	3, 4	74.5	1073	74.2	1077	74.6	1108
do	5, +	75.6	1206	76.4	1198	76.1	1177
Holstein	< 3	76.6	1214	76.4	1208	76.2	1215
do	3, 4	80.1	1362	79.7	1348	79.5	1341
do	5, +	81.7	1431	81.4	1421	81.4	1424
Jersey	< 3	67.3	819	67.1	819	67.4	840
do	3, 4	69.8	923	69.6	922	69.7	930
do	5, +	72.6	995	72.3	976	72.1	984

accordingly the g value for this group is $71.5 - 69.6 = 1.9$, or 2 as given in figure 2. The value of g for each of the other age-breed groups is computed in a similar way. The device of figure 2 thus affords a compact table for estimating initial live weight from a chest girth measurement, recognizing age-breed groups.³

DISCUSSION

The diagram of figure 3 is presented to explain in more detail the principles involved in the present girth-weight equation. In it $\log W$ is plotted against $\log G$ as a straight line for each of two groups. It is clear that if these two lines have the same slope (the same value of b in the girth-weight equation) they may be made to lie on a common straight line by shifting the horizontal scale; likewise for any number of group lines of the same slope. It is the function of g in the equation to take care of this horizontal shift. The principle involved is rigorously valid if a single known value of b applies to all groups. It may be practically valid by using a compromise value of b , as in the present treatment.

If b is chosen too large the equation will overestimate live weight at large girths and underestimate it at small girths. If b is chosen too small an opposite situation will prevail. From table 1 and figure 1 it appears that the observed b is not larger than 1.85 for the initial data of the Holstein and

³ Obviously age-breed differences in the girth-weight relationship must have an anatomical basis. The question arises, can g in the equation be based on some measurable dimension index (such as spring of rib) instead of age-breed, thus putting the weight estimate on a more accurate basis with respect to the individual cow? If such a dimension index is used to determine g the observations should be classified by this index (instead of age-breed) to determine the equation. That is, the index should be substituted for age-breed all the way through. The present use of age-breed is regarded as an expedient to be used until it can be replaced by a better system.

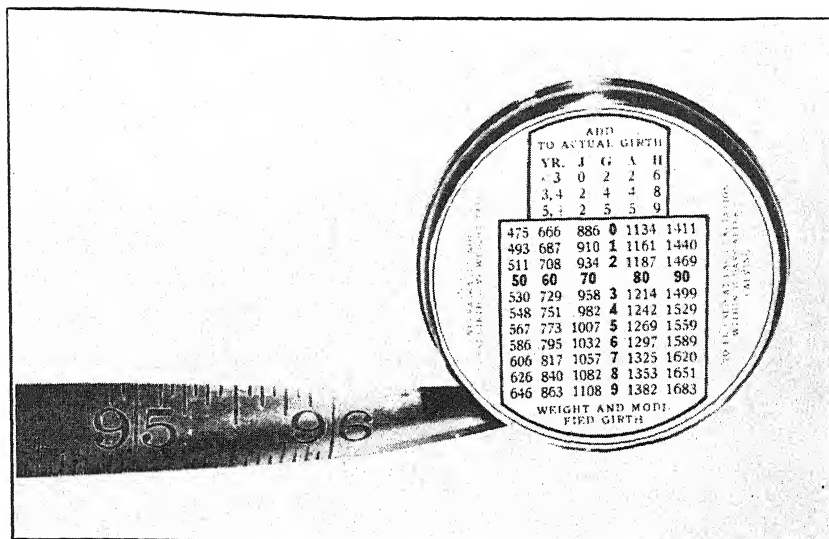


FIG. 2. Photograph of 96-inch steel tape rule and table for estimating initial live weight of cow from her chest girth. The tape has a J-shaped blade, $\frac{1}{2}$ inch wide. In operation the blade is withdrawn and the case end dropped over the back of the cow, thus flexing the blade which permits reaching the case from underneath the chest and completing the chest-girth measurement. (The blade does not flex freely under its own weight.) The tape is drawn under a force of about 3 pounds to encircle the chest in the region immediately back of the shoulders or region of smallest girth, and the girth read to the closest $\frac{1}{2}$ inch. This actual girth is then modified by addition of the age-breed factor indicated and the corresponding weight, in pounds, is read from the table, interpolating for the half inch, where necessary. *The table is not to be used until the actual girth has been modified as indicated.* Preliminary evidence indicates that the girth modifiers for Holstein cows should be used also for Brown Swiss and Milking Shorthorn cows. The tape pictured is a stock article of trade, except for the printed matter. It would be possible to incorporate the table on the blade of the tape, replacing the standard inch graduations with the appropriate weight figures, and supplementing the first end of the blade with an extension to take care of the girth modifier feature, thus providing a direct-reading girth-weight tape adapted to breed and age of cow.

Jersey breeds. The Ayrshire and Guernsey initial data suggest the exponent 2.00 rather than 1.85. If the present observations are representative of these two breeds the table of figure 2 (based on the exponent 1.85) will tend to overestimate weights at small girths and underestimate weights at large girths. The magnitude of this bias (if 2.00 is the correct exponent) is given by the fraction $[(G+g)/(\bar{G}+g)]^{.15}$. At the mean girth there is no bias; at $G+g=70$ and $\bar{G}+g=80$, which would be an extreme case, the correct (exponent 2.00) weight is .98 $[= (70/80)^{.15}]$ of the weight by figure 2 (exponent 1.85). That is, the weight is overestimated by 2 per cent at this point.

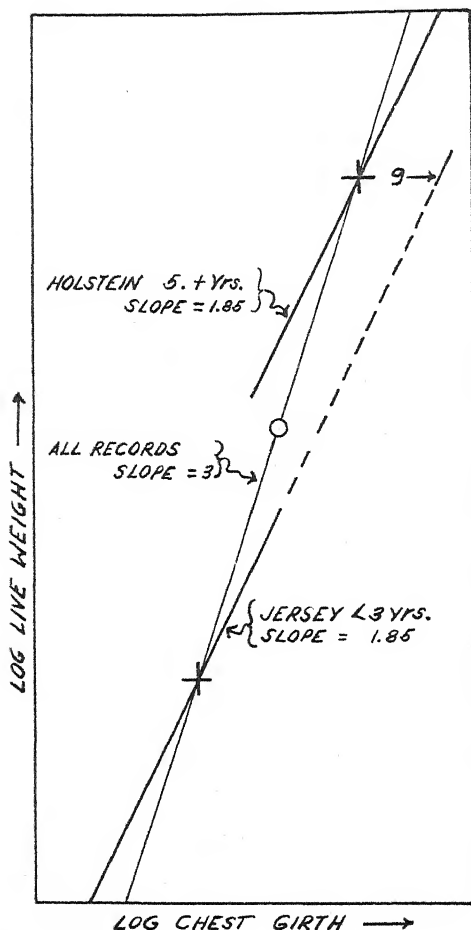


FIG. 3. Diagram to illustrate the principles involved in the girth-weight equation, $W = a(G + g)^b$. After selecting the compromise, $b = 1.85$, g is taken as zero for the Jersey < 3 years group and a is computed to make the equation line go through the observed mean girth and weight (cross) for this group. The observed mean weight (cross) of the Holstein 5, + years group is then shifted onto the equation by the term, g , and other groups are treated in a similar way. Any group may have an observed b (table 1) somewhat different than 1.85 and hence can be shifted onto the equation at only one point. The observed mean weight is used as this point.

If all groups are thrown together the regression turns on the total mean girth and weight (circle) and tends to go through or close to the group means (crosses). In the diagram it has a slope of 3 which is about what is found in actual lumped observations. This is in conformity with the 3d power rule relating linear dimensions and volumes. On the basis of the experimental evidence of the total data and the geometrical considerations what could be more natural than to say that the 3d power rule is a general law relating girth and weight? But we have seen such a law does not hold within groups—a striking demonstration that the regression within groups may be quite different than the regression in total. The lesson may be taken into many other data, for example, if “basal metabolism” is related to live weight including species from “mouse to elephant” a beautiful “3d power rule” is revealed. One should be on guard about presuming that the same rule

In a previous paper (5) live weight was estimated by the so-called New York tape, which is supposed to apply without regard to breed, age, stage of lactation, etc. The origin of this scale is unknown to the writers, but it conforms quite closely to a power equation with exponent 2.85. The difference between the scale of the New York tape and that of the Nebraska-Illinois tape, figure 2, is very serious as the following numerical comparison shows:

Chest girth, inches	50	60	70	80	90
Live weight, New York tape, pounds	388	656	1055	1502	1951
Live weight, { Holstein; < 3 years	586	795	1032	1297	
Neb.-Ill. tape, { Holstein; 5, + years		863	1108	1382	1683
(fig. 2), { Jersey; < 3 years	475	666	886	1134	
pounds { Jersey; 5, + years		708	934	1187	1469

As compared with the present scale, figure 2, the scale of the New York tape grossly overestimates the weight of large cows and grossly underestimates the weight of small cows. The magnitude of this bias may amount to 20 per cent or more.

A similar fault is bound to occur in any single scale derived from mixed breed, age, stage-of-lactation data. The fault arises in the fact that the regression of weight on girth is distinctly different within groups from what it is in total. This is especially prominent with respect to breed groups, less so with respect to age groups. The difficulty may be appeased by use of separate equations or by use of girth modifiers as in figure 2. Hopefully it may be overcome along the line suggested in footnote 3.

SUMMARY

An equation is derived for estimating initial live weight (live weight within 31 days after calving, in distinction to live weight at other or mixed stages of lactation) from chest girth. The equation is $W = .342 (G + g)^{1.85}$, where W is initial live weight in pounds, G is actual chest girth in inches and g is a girth modifier for age and breed of cow, being zero for Jersey cows under 3 years of age and ranging up to 9 for Holstein cows 5 years of age or older. The equation permits a compact girth-weight table which is imprinted on the case of a steel tape rule used to measure the chest girth. As compared with the present girth-weight scale the scale of the New York girth-weight tape grossly overestimates the weight of large cows and grossly underestimates the weight of small cows, as much as 20 per cent. This fault of the New York tape is inherent in any formula derived from mixed data, with respect to breed, age, stage of lactation, and gestation.

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FACTORS AFFECTING THE GAS CONTENT OF MILK¹

C. I. NOLL AND G. C. SUPPLEE

Borden Biological and Chemical Research Laboratories, Bainbridge, New York

The presence of oxygen, nitrogen and carbon dioxide as dissolved gases in milk has long been recognized. However, few papers have appeared on this subject since the studies of Marshall in 1902 (1). Most of the data published since that time have been primarily concerned with the effect of oxygen on milk constituents and the consumption of oxygen by bacteria. Little direct attention has been given to the effect of physical factors, temperature, pressure and light, and chemicals, on the gas content of milk. A systematic study of these factors as operative in the routine handling of milk and as a basis for potential improvement in operating practices has appeared desirable.

Before presenting experimental data, a brief review of the earlier work seems appropriate. The gas content of anaerobically drawn milk reported by Marshall (1), Van Slyke (2), Jackson (3), Frayer (4) and others, is summarized in table 1. (Recalculation of the original data to the volume per cent basis, that is volume of gas at 0° C., 760-mm. pressure per 100 ml. of milk has been made to facilitate interpretation and comparison.) It should be noted that the gas remaining after the absorption of oxygen and carbon dioxide, is called nitrogen by most of the investigators. This convention is used throughout the present work, but it is recognized that this

TABLE 1
Gas content of anaerobically drawn milk

Investigator	Oxygen	Nitrogen	Carbon dioxide	Total gas
	vol. %	vol. %	vol. %	vol. %
Hoppe*	0.14	1.37	1.65	3.00
Setchenow*	0.17-0.32	1.33-1.41	5.00- 6.71	6.67-8.29
Pfingler*	0.09-0.10	0.71-1.00	7.40- 7.66	8.47-8.49
Marshall	0.12-0.14	0.82-1.02	3.44- 4.96	4.40-6.10
Van Slyke	8.00-56.00
Jackson	25.80-28.70
Frayer	0.092	1.184	6.577	7.853

* Quoted by Marshall.

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may not be pure nitrogen since methane, ammonia and hydrogen may also be present; the nitrogen content could also be called "residual gas."

The change in the gas content of milk immediately upon contact with air, as in the milking process, is indicated by the data summarized in table 2. (Compare with table 1.) These results indicate a drop in the carbon dioxide content and an increase in the oxygen and nitrogen contents.

TABLE 2
Gas content of milk immediately after milking

Investigator	Oxygen	Nitrogen	Carbon dioxide	Total gas
	<i>vol. %</i>	<i>vol. %</i>	<i>vol. %</i>	<i>vol. %</i>
Thorner*	0.36-0.44	1.82-1.87	3.57-6.25	5.70-8.56
Marshall	0.86	1.71-2.19	2.93-3.78	5.50-6.84

* Quoted by Marshall.

Marshall (1) has shown that the oxygen and nitrogen content of milk may be increased to 1.15 and 2.47 volume per cent respectively, by aeration methods. The work of Van Slyke (2) has indicated that one-third of the carbon dioxide in milk exists as carbonic acid and two-thirds as bicarbonate. Jackson (3) has presented data indicating the carbon dioxide content of anaerobically drawn milk to be 25 to 28 volume per cent, which is approximately one-half that of blood plasma. He points out that this ratio compares well with the ratio between the total sodium and potassium in blood plasma and milk, and concludes that these elements are the principle binders of carbon dioxide in milk.

Sharp, Guthrie and associates (5, 6, 7) have shown a relationship between the oxygen content of milk, the development of oxidized flavor and the destruction of ascorbic acid; likewise, various data have been presented showing the oxygen content of milk throughout different phases of commercial handling and processing. A comprehensive report by Frayer (4) has correlated a decrease in the dissolved oxygen content of milk and an increase in the carbon dioxide content with increases in bacterial count; he also noted that the dissolved oxygen content of milk rapidly decreased when milk was exposed to sunlight.

While both Sharp and Frayer have shown the general effect of temperature on the gas content of milk, one of the objects of this paper is to show the direct relation between the amount of dissolved gas in milk, particularly oxygen, and the physical principles governing the solubility of a gas in a liquid. According to these principles, at constant pressure the solubility of a gas in a liquid is inversely proportional to the temperature.

EXPERIMENTAL

Since the plan of the work reported herein involved the correlation of analytical values for dissolved oxygen, nitrogen and carbon dioxide in milk

subjected to controlled physical treatment, the selection and adaptation of appropriate methods of analysis was of importance. The manometric procedure and calculation of results described by Van Slyke and Neill (8) for the successive determination of carbon dioxide, oxygen and nitrogen in blood, were employed throughout the work, with the exception that lactic acid was used for liberating carbon dioxide in lieu of sulfuric acid. An extended series of preliminary analyses showed that values for each of the gases determined on duplicate samples of milk readily checked well within 0.05 volume per cent.

The typical gas content of mixed milk as sampled from a reservoir conjoined with the usual piping, to the receiving vat in a commercial milk plant is shown in table 3; these results were accumulated over a period of several months. The results are in good agreement with those obtained by Sharp (7) and Frayer (4) from similar commercial milk (summarized in table 4) and should serve as adequate evidence concerning the normal gas content of milk as received by commercial milk handling establishments. In comparing these results with those in table 1, it is apparent that the oxygen and nitrogen contents are higher than those for anaerobically drawn milk, whereas the carbon dioxide content is lower; they are of the same order of magnitude as those shown to prevail immediately after milking (table 2).

TABLE 3
Gas content of mixed raw milk as received at a commercial milk plant

Sample No.	Oxygen	Nitrogen	Carbon dioxide	Total gas
	<i>vol. %</i>	<i>vol. %</i>	<i>vol. %</i>	<i>vol. %</i>
1c	0.48	1.32	4.02	5.82
56c	0.45	1.24	3.73	5.42
20c	0.59	1.38	4.07	6.04
44c	0.51	1.27	4.71	6.49
90c	0.45	1.18	4.30	5.93
108c	0.51	1.22	6.28	8.01
121c	0.54	1.26	3.95	5.75
145c	0.43	1.32	4.88	6.63
153c	0.51	1.35	4.74	6.60
196c	0.36	1.24	4.49	6.09
Minimum				
(63 samples)	0.30	1.18	3.44	4.92
Maximum				
(63 samples)	0.59	1.63	6.28	8.50
Average				
(63 samples)	0.47	1.29	4.45	6.21

It is apparent that oxygen and nitrogen are rapidly absorbed by milk upon exposure to air even as prevails during milking, with the consequent result that the amount of these two gases dissolved in milk at the time it reaches the processing plant is markedly different and higher than when drawn. The average value of 0.47 volume per cent oxygen found in com-

TABLE 4

Summarized comparison of the gas content of raw commercial mixed milk

Investigator	Oxygen (vol. %)			Nitrogen (vol. %)			Carbon dioxide (vol. %)		
	Max.	Min.	Ave.	Max.	Min.	Ave.	Max.	Min.	Ave.
Sharp	0.51	0.47	0.49
Frayer	0.74	0.20	0.43	1.48	1.17	1.37	4.50	2.78	3.63
Authors	0.59	0.30	0.47	1.63	1.18	1.29	6.28	3.44	4.45

mercial milk at an average temperature of about 15° C. is quite comparable to the value of 0.52 volume per cent found in stored distilled water at 24° C. It may be assumed, therefore, that a state of equilibrium in milk is approached and seemingly reaches consummation fairly rapidly. Critical values for the ratio of oxygen to nitrogen dissolved in water in equilibrium with air are 33.9:66.1. The ratio of oxygen to nitrogen calculated from the average values for milk as recorded above is 26.7:73.3, assuming the "residual gas" is entirely composed of nitrogen. This ratio would undoubtedly more closely approach the equilibrium ratio for water if the methods of analysis permitted a segregation of the values for true dissolved nitrogen and other possible constituents of the "residual gas," such as traces of ammonia, known to be present in fresh milk. These considerations would tend to give high "nitrogen" values. Likewise, it is probable that oxidation of certain milk components is initiated in the presence of dissolved oxygen and progresses continuously from the time the milk is drawn and proceeds concurrently with absorption of oxygen from the air. This contingency would tend to cause a lag in the oxygen equilibrium in milk as compared with water. These relationships exemplify the operativeness of well known physical principles governing the amount of dissolved oxygen in milk, and bring into bold relief the basic character of the problems involved in practical methods for the elimination or control of the oxygen content of milk as handled and processed under practical conditions.

THE EFFECT OF TEMPERATURE

The influence of different heat treating methods on the gas content of raw commercial mixed milk may be illustrated by the results obtained from short time-high temperature pasteurization equipment if provision is made for securing valid samples. The Trumbull Electro-Pure apparatus and the Flowing Film Electric Pasteurizer described by Supplee and Jensen (9) were used for obtaining data of this character. Milk samples from the Electro-Pure apparatus operated at different temperatures were collected in an atmosphere of nitrogen from a by-pass at the top of the heating unit without previous cooling. Notwithstanding rigid control of the method for obtaining completely filled sample containers, and appropriate sealing prior

to analysis, the momentary exposure of the hot milk to an atmosphere of nitrogen presumably permits some of the dissolved gases to escape. The results summarized in table 5 are averages of several determinations which show the effect of temperatures from 162° F. to 190° F. on the gas content of milk treated in this particular type of apparatus operated without the heat regenerator. The values for the oxygen content plotted against the pasteurization temperature are shown graphically in figure 1. The zero value for 212° F. is necessarily included as a point of reference. The straight line relationship shown by these data is in conformity with critical reference data for the solubility of oxygen in water over the same temperature range, thus confirming the general law governing the solubility of gases in a liquid as affected by temperature.

TABLE 5

Gas content of hot milk samples taken from an electro-pure pasteurizer immediately after pasteurization at various temperatures

Pasteurization temperature	Oxygen	Nitrogen	Carbon dioxide
° F.	vol. %	vol. %	vol. %
162	0.35	1.05	4.48
175	0.24	0.92	4.22
185	0.18	0.78	4.02
190	0.10	0.71	4.63

Further studies on the gas content of milk treated in the Electro-Pure Apparatus were carried out with the technique just described, but with the

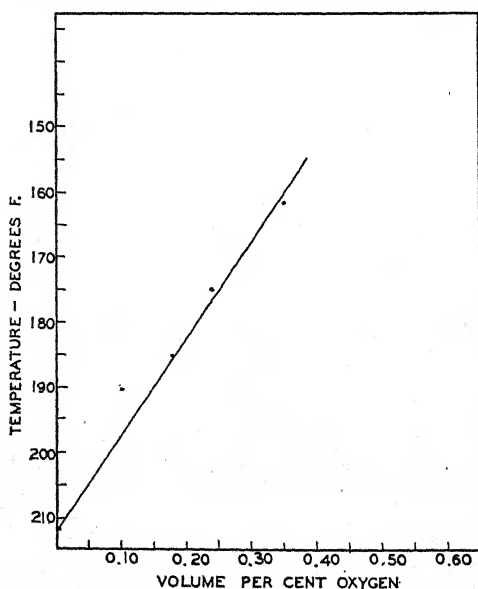


FIG. 1. Dissolved oxygen in hot milk direct from electro pure apparatus.

exception that all milks were pasteurized at 195° F. and subsequently cooled to 100° F. in an internal spiral condenser under conditions which permitted heating and subsequent cooling in a completely closed system. The data, summarized in table 6, show an average oxygen content of 0.23 volume per cent as compared with 0.10 volume per cent for the hot milk pasteurized at 190° F. but not subsequently cooled (table 5). The nitrogen content of the cooled milk is likewise significantly higher than was found in the hot milk at the same comparable pasteurization temperature. Since the collection and sealing of the samples for both series of determinations were carried out under the same rigidly controlled conditions, the lower oxygen and nitrogen values found in the hot milk momentarily exposed to nitrogen during collection of the samples, are interpreted as being due to the fact that the gas dissolved in the hot milk was dispelled immediately upon release from the closed system, whereas the hot milk subsequently cooled in a closed system could not dissipate the gas at the higher temperature.

TABLE 6

Gas content of milk pasteurized at 195° F. with an electro-pure pasteurizer and cooled to 100° F. in a closed system

Run No.	Oxygen	Nitrogen	Carbon dioxide
	<i>vol. %</i>	<i>vol. %</i>	<i>vol. %</i>
1	0.26	0.88	4.48
2	0.19	1.08	4.75
3	0.24	0.89	4.65
4	0.19	0.89	4.60
5	0.25	0.91	3.67
Average	0.23	0.93	4.43
Average of unheated samples	0.44	1.33	5.02

The gas content of milk as affected by treatment at 185° F. and subsequent cooling in air with the Supplee-Jensen equipment, is shown in the data summarized in table 7. These results were obtained from milk fed to the apparatus from an internal preheater, the milk going to the heating unit at 90° F. Samples for gas analysis were taken from the storage tank ahead of the preheater, as the milk left the heating unit at 185° F., and upon leaving the cooler at a temperature of 50° F. The results show substantially a 50 per cent decrease in oxygen content of the hot milk; an average value of 0.26 volume per cent as compared with 0.48 volume per cent in the raw milk, and a reabsorption on cooling in air to 0.35 volume per cent or about three-fourths of the amount originally present in the unheated milk. It will be noted that the oxygen content of the hot milk as it left the heating unit is substantially the same as that for the milk heated and cooled in the closed system (Electro-Pure Apparatus, table 6). Since the maximum heating temperature in both instances was of the same order of magnitude, 195° F.

and 185° F., the data are of particular interest in further correlating oxygen content of the milk with its temperature. However, the data presented in table 7 indicate rapid reabsorption of oxygen upon exposure to air at lower temperatures, as during cooling in ordinary atmosphere.

TABLE 7
Gas content of milk samples taken from a flowing film pasteurizer
(Flow rate—5 oz. per inch per minute)

Samples from	Milk temp.	Oxygen	Nitrogen	Carbon dioxide
	° F.	vol. %	vol. %	vol. %
Feed tank	68	0.48	1.28	3.98
Heating unit	185	0.26	0.65	2.45
Cooler	50	0.35	0.93	2.13

Comparing the foregoing results with those reported by Sharp (7) and Frayer (4) for the holding method of pasteurization (table 8) it will be noted that the oxygen content of the hot milk is of the order of magnitude which might be anticipated from the temperature (144° F.) to which the milk is subjected. Cooling to a low temperature over an external cooler causes reabsorption of oxygen to substantially the same, or possibly a higher level than has been reported for average unprocessed raw milk. The results reported herein and those recorded by Frayer show a drop in the carbon dioxide content of milk on heating, and a further decrease on cooling in air. This is readily understandable in view of the fact that the amount of carbon dioxide in the atmosphere under ordinary conditions is extremely small and accordingly its partial pressure is substantially below that required for saturation of the milk.

TABLE 8
Gas content of milk during the holding process of pasteurization (144° F.)

Origin of samples	Reported by Sharp	Reported by Frayer		Carbon dioxide
	Oxygen	Oxygen	Nitrogen	
	vol. %	vol. %	vol. %	vol. %
Before heating	0.77	0.557	1.287	3.944
Beginning of holding period	0.39	0.493	1.156	3.944
End of holding period	0.32	0.395	1.075	3.814
Bottom of external cooler (40–47° F.)	0.55	0.522	1.019	2.337

THE EFFECT OF PRESSURE

It is common knowledge that by increasing the pressure of a gas over a solution the weight of the dissolved gas in the solution is increased; this is expressed quantitatively by Henry's Law for the solubility of gases in liquids. The experimental procedures used in studying the effect of pres-

sure on the gas content of milk were adapted for comparing methods for the removal of dissolved gas, particularly oxygen. The most general procedure for the removal of gases from liquids involving the application of pressure principles, is to subject the liquid to a vacuum treatment. Other methods may be described as displacement procedures based on the removal of a particular gas by flushing with a different gas. Specific data involving each of these methods as applied to milk, singly or in combination, are presented hereinafter. Specimen data showing the rate and degree of reabsorption of gases by deaerated or deoxygenated milk as influenced by the pressure of the gas above the solution are also presented.

It has been shown previously that raw commercial milk contains substantially 0.47, 1.29 and 4.45 volume per cent respectively of oxygen, nitrogen and carbon dioxide (table 3); these ratios representing approximate equilibrium with the partial pressure of the oxygen and nitrogen in the air over the milk. Such milk may be substantially completely deoxygenated and its nitrogen and carbon dioxide contents markedly reduced by vacuum treatment applied in two different ways, which will be designated as methods 1 and 2.

Method 1 involved heating milk to 185° F. and rapidly cooling to 100° F. with the Flowing Film Electric Pasteurizer to which reference has previously been made (9), the heating and cooling being carried out in an atmosphere of nitrogen. The treated milk was passed directly without exposure to air into an appropriate receptacle maintained at 24 to 29 inches vacuum. Method 2 involved deaeration with the apparatus and procedure described by Sharp, Guthrie and Hand (7). The milk entering the apparatus at 105° F. was exposed momentarily to 29 inches vacuum in the deaeration chamber, the temperature dropping to 75° F. Typical results obtained from each of these rapid treating methods involving the use of vacuum are shown in table 9, from which it will be noted that the oxygen remaining in the milk after treatment by either method is practically nil.

TABLE 9
Gas content of milk subjected to vacuum treatment

Method	Vacuum	Oxygen	Nitrogen	Carbon dioxide
	<i>inches</i>	<i>vol. %</i>	<i>vol. %</i>	<i>vol. %</i>
No. 1—Run 1	24.0	0.10	0.40	2.44
No. 1—Run 2	29.0	0.03	0.36	1.70
No. 1—Run 3	29.0	0.01	0.36	1.67
No. 2—Deter. 1	29.2	0.02	0.19	2.01
No. 2—Deter. 2	29.2	0.01	0.19	1.96
No. 2—Deter. 3	29.2	0.06	0.26	2.96

Other methods used for varying the partial pressure of a gas over a solution and consequently effecting removal of gas from solution involve dis-

placement of a particular gas with a different gas. Experimental work embodying this principle consisted in flushing milk with air, nitrogen or carbon dioxide under atmospheric pressure, a positive pressure of 100 pounds and under vacuum. Facilities were readily provided for a comparison of these procedures by an appropriate assembly and manipulation of items of equipment and accessories obtained from the American Instrument Company particularly designed for the handling and control of gas under positive or negative pressures. Typical detailed and summarized data from this series of experiments are shown in the accompanying tables 10 to 13 which show the following relationships: Flushing milk with oxygen at 25 inches vacuum at 11–13° C. for as long as three hours (table 10) causes no reduction in the dissolved oxygen content of milk, as may be expected, but does materially reduce the dissolved nitrogen and carbon dioxide content. Milk flushed with air at 25 inches vacuum at 11° C. for 60 minutes shows a substantial reduction in the oxygen, nitrogen and carbon dioxide content, which with the exception of carbon dioxide, is not substantially reduced upon continued flushing for a two-hour period (table 11). Flushing with air at 25 inches vacuum for 60 minutes at variable temperatures from 11–49° C. causes a reduction in each of the three gases commensurate with the temperature of the milk, the period of treatment and degree of vacuum being constant; the amount of dissolved oxygen remaining after flushing at the highest temperature is practically nil.

Table 12 shows the comparative results obtained by flushing with nitrogen at 100 pounds pressure, at atmospheric pressure, and at 25 inches vacuum at a temperature of 8 to 11° C., from which it will be noted that the dissolved oxygen is reduced to a low value after 40 to 60 minutes. The data also show the anticipated increase in dissolved nitrogen content, saturation or supersaturation with this gas at atmospheric or positive pressure, and the saturation equilibrium at 25 inches vacuum at a consistently lower concentration.

Table 13 shows the results obtained by flushing milk with carbon dioxide at 25 inches vacuum at 13 to 16° C. from which it will be noted that the dissolved oxygen content is reduced to the same low value obtained by flush-

TABLE 10
Gas content of milk flushed with oxygen at 25 inches vacuum
(Temperature—11–13° C.)

Flushing time	Oxygen	Nitrogen	Carbon dioxide
<i>min.</i>	<i>vol. %</i>	<i>vol. %</i>	<i>vol. %</i>
0	0.43	1.25	4.44
90	0.40	0.29	2.82
180	0.38	0.19	1.60
270	0.42	0.34	0.66
360	0.43	0.28	0.49

ing with nitrogen. The dissolved nitrogen content is likewise reduced to a low value whereas, the carbon dioxide content is markedly increased, seemingly to the saturation level of approximately 33 volume per cent.

TABLE 11
Gas content of milk flushed with air at 25 inches vacuum

Flushing time	Temp. of milk	Oxygen	Nitrogen	Carbon dioxide
<i>min.</i>	<i>°C.</i>	<i>vol. %</i>	<i>vol. %</i>	<i>vol. %</i>
0	0.48	1.22	5.03
60	11	0.16	0.48	3.79
120	11	0.17	0.45	2.30
0	0.50	1.25	5.40
60	11	0.16	0.47	4.10
0	0.50	1.29	5.11
60	33	0.06	0.30	1.90
0	0.48	1.32	4.94
60	49	0.03	0.24	0.36

TABLE 12
Gas content of milk flushed with nitrogen at atmospheric pressure, 25 inches vacuum and 100 lbs. pressure (Temperature—8–11° C.)

Flushing time	Atmospheric pressure			25 inches vacuum			100 lbs. pressure		
	O ₂	N ₂	CO ₂	O ₂	N ₂	CO ₂	O ₂	N ₂	CO ₂
<i>min.</i>	<i>vol. %</i>	<i>vol. %</i>	<i>vol. %</i>	<i>vol. %</i>	<i>vol. %</i>	<i>vol. %</i>	<i>vol. %</i>	<i>vol. %</i>	<i>vol. %</i>
0	0.51	1.32	4.13	0.54	1.32	4.14	0.51	1.27	4.43
20	0.18	0.51	3.93
30	0.28	1.63	3.35
40	0.05	0.41	3.32
60	0.05	1.83	2.89	0.05	0.52	2.50
120	0.04	1.72	2.18	0.00	0.42	1.56	0.02	1.87	1.76
150	0.00	0.48	1.07
180	0.04	1.83	1.35	0.01	0.51	0.99
240	0.00	1.80	1.14	0.02	0.61	0.64
300	0.01	1.82	0.64	0.00	0.60	0.39
420	0.01	1.88	0.24

TABLE 13
Gas content of milk flushed with carbon dioxide at 25 inches vacuum—average of four runs (Temperature—13–16° C.)

Flushing time	Oxygen	Nitrogen	Carbon dioxide
<i>min.</i>	<i>vol. %</i>	<i>vol. %</i>	<i>vol. %</i>
0	0.47	1.26	4.10
90	0.07	0.22	28.20
180	0.03	0.19	31.44

Failure to reduce the carbon dioxide concentration to as low a value as was obtained for oxygen and nitrogen, by flushing with air or nitrogen is in agreement with the general observations of Van Slyke (2) who found it extremely difficult completely to remove carbon dioxide from milk. This gas is reactive and forms ionizable solutions (carbonic acid) in water and also, according to the work of Jackson (3) and Van Slyke (2) previously cited, a portion of the carbon dioxide in milk is presumably bound as bicarbonate, a condition further accentuating difficulty in complete removal by the methods employed. It is to be noted that irrespective of the gas flushing methods used with or without vacuum, the dissolved nitrogen content of the milk was not reduced below 0.19 volume per cent. An explanation for failure to remove this "residual nitrogen" increment cannot be given at the present time, unless the contingent possibilities referred to earlier in this paper inherently prevail in the method of analysis employed.

By applying the mathematical expression of Henry's Law to the data in table 10, assuming little or no absorption of oxygen during collection of the samples, an approximate calculation of the oxygen content of milk in equilibrium with air at atmospheric pressure (approximately 740 millimeters at the authors' laboratory) can be made. This calculated value amounts to 0.54 volume per cent at 12° C. which agrees well with the average value of 0.47 volume per cent oxygen for milk collected at about 15° C. (table 3). If this slight temperature difference is taken into account, it is apparent that the samples of raw mixed milk were saturated with the oxygen to about 87 per cent of the final equilibrium value potentially attainable at this temperature.

The application of Henry's Law for the removal of gases dissolved in milk may be further illustrated with data obtained by pasteurizing milk with the Flowing Film Electric Pasteurizer (9) operated in air and in an atmosphere of nitrogen. The apparatus, including the heating unit and cooler, was inclosed in a manner which permitted a stream of air or nitrogen to be passed over the milk film during heating and cooling in a direction counter to the flow of the milk film. The average results for three complete comparisons at different temperatures are shown in table 14, all samples being taken after cooling. Treatment in air shows no significant reduction in the oxygen content although the nitrogen and carbon dioxide contents are measurably reduced. However, the oxygen content of the samples pasteurized in nitrogen is lowered by approximately 50 per cent which is in agreement with the results obtained from samples of hot milk as previously reported (tables 6 and 7). Substantially little or no oxygen is reabsorbed during cooling in an atmosphere of nitrogen, the oxygen pressure over the milk being nil. The oxygen content of the milk pasteurized at different temperatures is not commensurate with the temperature employed. This may probably be explained by the extremely low and uniform partial pressure of the oxygen at the

surface (at elevated temperatures oxygen is dispelled from the milk into the nitrogen atmosphere at the interface) resulting from the flowing nitrogen, irrespective of the temperature. However, it is recognized that the short time during which the milk is above 145° F., only 0.7 to 0.8 seconds, makes it unlikely that a final equilibrium was reached in all instances.

TABLE 14

Gas content of milk samples pasteurized with flowing film heater in air and nitrogen at various temperatures

(Temperature of samples taken from cooler—52–55° F.)

Pasteurizing temperature	Pasteurized in air			Pasteurized in nitrogen		
	O ₂	N ₂	CO ₂	O ₂	N ₂	CO ₂
°F.	vol. %	vol. %	vol. %	vol. %	vol. %	vol. %
Control	0.35	1.27	4.45	0.35	1.27	4.55
162	0.31	1.06	3.92	0.18	1.16	3.85
172	0.32	1.11	3.84	0.21	1.12	3.66
180	0.32	1.02	3.71	0.16	1.16	3.51
185	0.34	1.13	3.25	0.15	1.19	3.12

Although the amount of oxygen reabsorbed by deoxygenated milk is dependent chiefly on the temperature of the milk and the partial pressure of the oxygen in the contacting atmosphere, the rate of reabsorption is chiefly dependent upon the relative surface exposure per unit of volume. The effect of this factor is well illustrated by the following series of experiments: Milk previously deoxygenated to a uniformly low value by the different methods heretofore described, was exposed to air under integrally controlled conditions by flowing it successively over 1, 2 or 3 eight-inch miniature corrugated coolers contiguously arranged to permit the film to pass from one cooler to another without significant interruption of the continuity of the flow. The deoxygenated milk was introduced in the system at 50° F. and maintained at this temperature during exposure to air, as a flowing film with a capacity of four ounces per inch per minute. The exposure time per unit was substantially two seconds. The results (table 15) show a rapid reabsorption of oxygen during momentary exposure to the atmosphere with a proportionate increase as the time of exposure is increased. The amount of oxygen absorbed does not appear to be influenced by the original nitrogen concentration. This is in agreement with the principle that the solubility of a gas depends on the partial pressure of the same gas over the solution and is independent of the presence of other dissolved gases. Calculation of the oxygen to nitrogen ratios for each of the milks progressively exposed to air under these conditions, shows that the amount of each of the gases going into solution gradually approaches the true solubility ratio of oxygen to nitrogen taken up by water from air. The change in the carbon dioxide values are smaller but are in the proper direction as is to be expected by the low partial pressure of this gas in air and its low initial concentration in the milk used.

TABLE 15

Gas content of deoxygenated and deaerated milk after exposure to air at constant temperature under controlled conditions

Previous treatment	Oxygen vol. per cent aeration time (sec.)				Nitrogen vol. per cent aeration time (sec.)				Carbon dioxide vol. % aeration time (sec.)			
	0	2	4	6	0	2	4	6	0	2	4	6
Vacuum flushed with nitrogen ...	0.06	0.13	0.21	0.31	1.58	1.58	1.52	1.52	1.59	1.60	1.54	1.44
Vacuum method No. 1	0.02	0.11	0.22	0.27	0.36	0.51	0.64	0.73	1.69	1.63	1.66	1.57
Vacuum method No. 2	0.04	0.15	0.23	0.27	0.81	0.90	0.96	1.03	2.53	2.80	2.45	2.75

It is apparent from the foregoing data that changes in the gas content of milk during various processing procedures are dependent upon the physical laws governing the solution of gases in liquids. It is also shown that substantially all of the oxygen may be removed from milk by subjecting it to vacuum treatment, flushing with other gases, or a combination of these methods. However, if milk of low oxygen content is exposed to air, oxygen is rapidly reabsorbed, the amount being dependent on the temperature and the partial pressure of the gas over the milk; the rate of reabsorption depending upon the extent of the surface exposed. These results are of practical interest in view of the work of Sharp and associates (5, 6, 7) on the relationship between the oxygen content of milk and the destruction of ascorbic acid therein.

THE EFFECT OF LIGHT AND THE OXIDATION OF ASCORBIC ACID ON THE GAS CONTENT OF MILK

The data presented thus far have been concerned primarily with the effect of physical factors of temperature and pressure on the gas content of milk. Another factor which affects the dissolved oxygen content is the oxidation of the ascorbic acid inherently present in milk or that which may be added. Since this oxidation process can readily be traced as a quantitative chemical reaction, ascorbic acid may be considered as a chemical reagent for the removal of dissolved oxygen from milk, and conversely the absence of oxygen may be considered as a prerequisite for preventing the destructive oxidation of this factor. Sharp (5), Guthrie (6) and Hand (10) have disclosed the role of oxygen in relation to the ascorbic acid content of milk with particular emphasis on the effects of light, heat, enzymes, and metals as catalysts affecting the rate of oxidation. The primary purpose of the present work is to emphasize the role of ascorbic acid as a deoxygenating agent and to show how this reactive property may be controlled notwithstanding the catalytic effect of light, heat and metals.

In order to facilitate these studies it was first necessary to establish a working procedure whereby the relationship between the quantitative oxidation of ascorbic acid and its rate of oxidation could be correlated with the degree and rate of utilization of the dissolved oxygen. The ascorbic acid determinations were made by the rapid titration method described by Sharp (11). The gas analyses were made by the same methods employed in the previous studies. Since light is known to catalyze the destruction of ascorbic acid in the presence of oxygen, a standardized irradiation procedure was developed which involved exposure of the samples to a "C" carbon arc operated at 50 watts and 60 amps., at a distance of 36 inches. The irradiation was carried out in completely filled cylindrical bottles of flint glass of uniform size and shape rotating at uniform speed on a vertical wheel, thus permitting duplicable exposure conditions from time to time.

The correlation between the destruction of ascorbic acid in milk and the degree of reduction of the dissolved oxygen was determined in two different ways, which may be briefly described as follows: Normal fresh milk was exposed to light under the conditions described above until all the ascorbic acid had been destroyed. (A 30-minute exposure period was found to be sufficient.) The milk was then aerated to assure a normal oxygen content. To this ascorbic acid-free milk there was added approximately 200 mgs. of pure ascorbic acid per liter. Following this addition the ascorbic acid titration value was found to be 195 mgs. per liter and the dissolved oxygen content 0.44 volume per cent. To another sample of normal fresh milk not previously exposed to light to destroy its inherent ascorbic acid content, there was added 100 mgs. of ascorbic acid per liter. Following this addition the ascorbic acid titration showed 114 mgs. per liter and its dissolved oxygen content was 0.53 volume per cent. Each of these samples was then exposed to the standard irradiation treatment for sixty minutes and one hundred eighty minutes, respectively. Table 16 shows the comparative results obtained from each of these samples before and after irradiation, from which it will be noted that the sample containing 195 mgs. ascorbic acid per liter before irradiation had decreased to 146 mgs. at the end of a 60-minute exposure period, showing a loss of 49 mgs. of ascorbic acid. Paralleling this reduction in ascorbic acid, the dissolved oxygen content decreased from 0.44 volume per cent to 0.03 volume per cent. The sample containing 114 mgs. of ascorbic acid per liter prior to irradiation, including the 100 mgs. added and the 14 mgs. inherently present in the milk, showed a reduction to 63 mgs. per liter after a three-hour irradiation period, or a loss of 51 mgs.; the dissolved oxygen content was concurrently reduced from 0.53 volume per cent to 0.01 volume per cent. No measurable difference was found in the nitrogen and carbon dioxide contents of either of the two samples of milk before and after irradiation.

TABLE 16

Gas and ascorbic acid content of milk containing added ascorbic acid before and after irradiation

Sample	Irradiation time	Ascorbic acid	Oxygen	Nitrogen	Carbon dioxide
	<i>min.</i>	<i>mg./l.</i>	<i>vol. %</i>	<i>vol. %</i>	<i>vol. %</i>
Raw milk previously irradiated plus added ascorbic acid	0	195	0.44	1.15	2.90
	60	146	0.03	1.23	2.87
Raw milk plus added ascorbic acid	0	114	0.53	1.29	3.59
	180	63	0.01	1.27	3.65

Since the reduction in ascorbic acid content was constant irrespective of the amount present and since the dissolved oxygen content of each sample had been exhausted during irradiation and seemingly within sixty minutes, it may be assumed that the limiting reactive substance was oxygen. Appropriate calculations show that 1.4–1.6 atoms of oxygen disappeared concurrently with the destruction of one molecule of ascorbic acid. Repeated determinations of this value using different milks gave results varying between 1.3 and 1.7 atoms of oxygen per molecule of ascorbic acid with an average value of 1.49 for seven determinations. These results are considered in excellent agreement with the range of values reported by Sharp (12) (1.3 and 1.7) and the average value of 1.4 as reported by Hand (13).

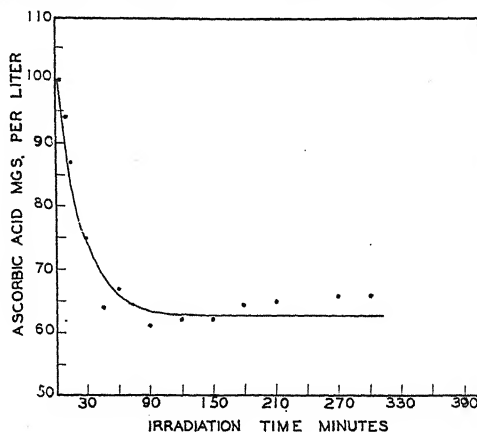


FIG. 2. Rate of destruction of ascorbic acid in milk exposed to intense light.

In view of the fact that further destruction of ascorbic acid could not be brought about by continuing the irradiation period beyond 60 minutes, it was desired to determine the rate of destruction within this period. For this purpose, milk pasteurized in an Electro-Pure apparatus at 195° F. was subsequently aerated to reestablish a normal dissolved oxygen content and 100 mgs. per liter ascorbic acid added. The ascorbic acid titration value follow-

ing this addition showed 105 mgs. per liter. Numerous subsamples of this milk were subjected to the irradiation treatment, individual sample containers being removed from the influence of the light at intervals up to 300 minutes. The ascorbic acid values obtained at the various intervals are shown in table 17 and graphically recorded in figure 2. The sharp initial drop in the curve with subsequent leveling off after approximately 45 minutes clearly show the character and rate of the reaction. The log. of the ascorbic acid concentration as a function of exposure time up to 45 minutes (fig. 3) shows a straight line indicating that destruction of ascorbic acid under these conditions is a first order reaction. This agrees with the work reported by Kon (14) on the destruction of ascorbic acid in raw milk exposed to sunlight.

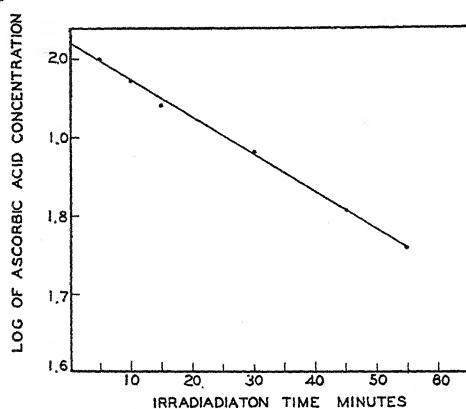


FIG. 3. Rate of destruction of ascorbic acid in milk. (Log of concentration as a function of irradiation time.)

TABLE 17

*The rate of destruction of ascorbic acid by light, in milk pasteurized at 195° F.
(Ascorbic acid added = 100 mg. per liter)*

Exposure time	Ascorbic acid	Log. ascorbic acid conc.	Velocity constant K
<i>min.</i>	<i>mg./l.</i>		
0	105	2.02
5	100	2.00	0.0098
10	94	1.97	0.0111
15	87	1.94	0.0126
30	75	1.88	0.0112
45	64	1.81	0.0110
60	67
90-300	65*

* Average of 9 additional determinations at 30 minute intervals.

The velocity constants (table 17) calculated from the equation for a first order reaction ($K = 0.009-0.012$) indicate that the reaction rate is only about one-sixth that found by Kon ($K = 0.05$ to 0.072). This difference may be due

to a difference in the catalytic effect of the light source, possible variation in copper contamination, or to the absence of catalyzing enzymes in the milk pasteurized at 195° F. Undoubtedly the light source is of importance in affecting the velocity of the reaction, as shown by the following summary of data. Velocity constants determined in a similar manner but wherein 100-watt Mazda lamps were used in lieu of the carbon arc, were found to be of a magnitude of $K = 0.0015-0.0020$, or about one-sixth the values obtained for the more reactive light source, other conditions being the same.

ABSENCE OF DISSOLVED OXYGEN, A PREREQUISITE FOR THE CONSERVATION
OF ASCORBIC ACID IN MILK

That the conservation of ascorbic acid in milk is predicated primarily on the absence of dissolved oxygen, may readily be demonstrated by simple but well-controlled experiments. Guthrie, Sharp and Hand (6) have shown such to be the case and further confirmatory data are presented hereinafter. Raw commercial milk having a dissolved oxygen content of 0.51 volume per cent and an ascorbic acid titration value of 15 mgs. per liter, was flushed with air in the absence of light for 90 minutes reducing the oxygen content to 0.20 volume per cent; the ascorbic acid value remained at 15 mgs. per liter. Upon exposure of this milk to the irradiation conditions previously described for 30 minutes, the ascorbic acid was reduced to zero. A parallel sample of mixed raw milk with a dissolved oxygen content of 0.47 volume per cent and an ascorbic acid titration value of 14 mgs. per liter, was flushed in the absence of light for 90 minutes with carbon dioxide, reducing the oxygen content to 0.01 volume per cent, causing no reduction in the ascorbic acid content. This milk with negative oxygen content, upon irradiation for 30 minutes, likewise showed no reduction in its ascorbic acid value. A further sample of fresh raw milk with a normal dissolved oxygen content of substantially 0.47 volume per cent and 14 mgs. ascorbic acid per liter flushed with nitrogen in the absence of light for 90 minutes showed an oxygen content of 0.02 volume per cent and no reduction in ascorbic acid value. The milk thus treated and subsequently irradiated for 30 minutes showed a reduction of only 1 mg. ascorbic acid per liter with no further decrease during an exposure period of 120 minutes. Even the addition of copper, in the amount of 1 mg. per liter as the acetate or lactate, to milk deoxygenated to a low level of 0.02-0.03 volume per cent by one or more of the methods mentioned heretofore, caused a minor reduction of only a few milligrams of ascorbic acid per liter upon exposure for 30 minutes to the intense irradiation employed throughout this work.

Probably a more readily visualized application of the foregoing principles is shown by the following series of experiments designed to determine the degree of conservation of ascorbic acid which would prevail in deoxygenated milk as delivered to and handled by the consumer. Milk of low

oxygen content processed by the deaeration method employed by Sharp and associates and milk of similar low oxygen content obtained by nitrogen flushing methods described herein was run into standard quart milk bottles without particular precautions for preventing exposure to air. The filled bottles were fitted with caps and hoods; one-half of the bottles were stored at ordinary refrigerator temperature for a period of 72 hours, individual bottles being withdrawn at intervals for the determination of dissolved oxygen and ascorbic acid. A parallel group of similarly bottled samples was handled in the same manner but with the exception that subsequent to filling and capping, caps were removed and approximately one-half the milk poured from the bottle. Caps without hoods were replaced and these partially filled bottles with an air column above the milk, were stored under the same conditions as the completely filled bottles; individual bottles being used at intervals for examination. There was no decrease in the ascorbic acid content in the filled, capped and hooded bottles, nor was there any increase in the dissolved oxygen content during a period of 72 hours under ordinary refrigerator storage conditions. These results are to be contrasted with those from the partially filled bottles wherein the dissolved oxygen content of the milk increased from substantially zero to 0.26 volume per cent with a corresponding reduction in ascorbic acid content. Storage periods beyond 24 hours did not cause a significant increase in the dissolved oxygen content, but a decrease in the ascorbic acid progressed constantly to substantially 30 per cent of the original value or the value maintained in the filled, capped and hooded bottles (table 18).

TABLE 18
Oxygen and ascorbic acid content of bottled deoxygenated milk
(Bottling and storage procedure)

Storage condition	Storage time	Oxygen	Ascorbic acid
	<i>hr.</i>	<i>vol. %</i>	<i>mg./l.</i>
Full bottles capped and hooded	0	0.04	12
	24	0.07	10
	48	0.06	10
	72	0.03	11
Half filled bottles, capped	0	0.04	12
	24	0.26	8
	48	0.25	5
	72	0.25	4

The operability of the physical principles, temperature and pressure, as affecting the gas content of milk and its relation to the stability of ascorbic acid is also shown by another series of experiments which take into account the essential feature of sterilization as prevails in the preparation of commercial evaporated milk. Raw milk and milk pasteurized at 185° F. in the Electro-Pure equipment, then homogenized, was subjected to the following

TABLE 19
Gas and ascorbic acid content of processed milk before and after sterilization

Milk	Before sterilization				After sterilization				After 36 days storage	
	Oxygen	Nitrogen	Carbon dioxide	vol. %	Oxygen	Nitrogen	Carbon dioxide	vol. %	Oxygen	Ascorbic acid
Raw	0.47	1.63	6.22	vol. %	0.27	1.51	vol. %	vol. %	vol. %	mg./l.
" *	0.38	1.37	5.80		0.02	1.76	6.95	0.00	0.00	4
" †	0.02	1.35	1.69		0.00	1.72	6.35	0.00	0.00	7
							2.51	0.00	0.00	22
Past. and homogenized	0.66	1.56	2.88		0.40	1.66	3.76	0.00	0.00	1
" *	0.36	1.68	3.03		0.02	1.94	3.88	0.00	0.00	7
" †	0.02	1.60	1.60		0.00	1.82	2.22	0.00	0.00	16

* Partially deoxygenated.

† Deoxygenated by vacuum flushing with nitrogen.

treatments: One portion received no further treatment; a second portion was partially deoxygenated; and a third portion was fully deoxygenated by nitrogen flushing methods heretofore described. Each lot was placed in tin cans, hermetically sealed and subjected to the usual sterilization temperatures prevailing in the preparation of evaporated milk. Table 19 shows the significant interrelationship of the dissolved gas content of each of these milks before and after sterilization correlated with the ascorbic acid content following the heat treatment. The undeoxygenated milk has a residual ascorbic acid content, following sterilization and storage, of only one to four milligrams per liter; the partially deoxygenated milk shows an ascorbic acid value of 7 milligrams per liter, whereas the fully deoxygenated but previously pasteurized milk has a value of 16 milligrams per liter and the fully deoxygenated previously unpasteurized and unhomogenized milk has a value of 22 milligrams per liter. It will also be noted that there is a reduction in the dissolved oxygen content during sterilization and subsequent storage and that the degree of reduction seems to follow the decrease in ascorbic acid. Obviously, in those samples where little or no dissolved oxygen was present prior to sterilization, no significant reduction could be detected and in such instances there has been no significant reduction in ascorbic acid.

The particular significance of this series of data is the fact that in the absence of dissolved oxygen, ascorbic acid in milk is stable even at the high temperature necessary for complete sterilization.

SUMMARY

1. The average gas content of mixed raw milk as received at a commercial milk plant over a period of several months was found to be 0.47 volume per cent oxygen, 1.29 volume per cent nitrogen and 4.45 volume per cent carbon dioxide.

2. The effect of temperature and pressure on the gas content of the milk is shown to follow the well-known physical laws governing the solubility of gases in liquids.

3. The oxygen content of hot milk is reduced about 50 per cent at the high temperatures prevailing in short-time pasteurization methods, however, upon subsequent cooling a substantial amount of oxygen is reabsorbed, the degree of reabsorption depending upon the prevailing conditions during cooling; oxygen may be reabsorbed to a higher level than prevailed in the unpasteurized milk if a large surface per unit volume is exposed to air during cooling.

4. Milk may be deoxygenated by subjecting to an appropriate vacuum treatment, flushing with other gases, or a combination of these principles; efficiency of the particular method employed is increased at elevated temperatures.

5. Ascorbic acid may be considered a reactive substance consuming oxygen dissolved in milk. It may be employed as a means of deoxygenating milk and conversely its stability or preservation in milk is dependent upon the absence of dissolved oxygen.

6. In the absence of dissolved oxygen ascorbic acid in milk is stable to intense light for long periods, to the oxidizing effect of copper salts, and to high temperatures in excess of 212° F. as employed for complete sterilization. Light, heat and metallic contamination are considered as catalytic agents which accelerate the oxidation of ascorbic acid in the presence of dissolved oxygen, but are seemingly ineffective in the absence of oxygen.

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THE BACTERIOLOGY OF BRICK CHEESE. I. GROWTH AND ACTIVITY OF STARTER BACTERIA DURING MANUFACTURE¹

JOHN C. GAREY,² EDWIN M. FOSTER,³ AND WILLIAM C. FRAZIER

Department of Agricultural Bacteriology, University of Wisconsin, Madison

Before 1930 Brick cheese received little attention from research workers, but during the past decade the results of several investigations on methods of manufacture have been published (2, 3, 4, 7, 8, 9, 10, 11, 12, 13, 14, 16), nearly all on the effects of various factors upon the final quality of the cheese, with little information on bacteriological aspects. The purpose of this investigation was to obtain a better understanding of the growth and activity of different bacteria, used alone and in various combinations, in Brick cheese during its manufacture. *Streptococcus lactis* and *Streptococcus thermophilus* were selected, because they are used commonly in the making of Brick cheese.

METHODS

Standard methods were used in the sampling of milk and starters (15). Samples of curd and of curd and whey were collected and prepared according to Frazier and co-workers (5) and Burkey (1). Bacterial counts were made by the direct microscopic method of Frazier *et al.* (5), and by cultural methods to be described.

The cultural medium for counting the starter bacteria had the following composition:

Carrot extract ⁴	100 ml.
Liver extract ⁵	100 ml.
Difco peptonized milk	10 gm.
Difco Neopeptone	5 gm.
Washed agar	11 gm.
Distilled water	800 ml.

Sodium hydroxide to pH 7.0 \pm 0.1

Freshly prepared medium, never over four days old, was used in all experiments. The diluted samples were placed into the tubes of 10 ml. of

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² Now in the Department of Bacteriology, University of Illinois, Urbana.

³ Now in the Department of Bacteriology, University of Texas, Austin.

⁴ Carrot extract: add one liter of water to one pound of ground carrots; heat for one hour in steamer; filter through cheesecloth; bottle and sterilize in autoclave.

⁵ Liver extract: add one liter of water to one pound of ground fresh beef liver; heat to 70° C., and hold for thirty minutes; filter through cheesecloth; bottle and sterilize in the autoclave.

melted agar at 45° C. and were mixed thoroughly with the agar. The tubes of solidified agar were incubated for four days in thermostatically controlled water baths. Garey (6) established that it was possible to separate quantitatively *Str. lactis* from *Str. thermophilus* by incubation at 22° C. and at 47° C. The former temperature inhibited the multiplication of *Str. thermophilus* but not of *Str. lactis*; while at the latter temperature the reverse occurred.

The method for the manufacture of Brick cheese was that described by Wilson and Price (16), and by Langhus (11) and will be referred to as the conventional method. Moisture determinations on the cheese were made by the procedure recommended by Wilster *et al.* (17).

The milk was that delivered by one patron of the University of Wisconsin dairy and was of excellent quality; the methylene blue reduction time was never less than seven hours, and the total number of bacteria, determined culturally, always was less than 2,000 per ml. The fat content of the milk ranged between 3.2 and 3.7 per cent. When pasteurized milk was used, pasteurization at 142° F. for 30 minutes was carried out in the cheese vats. Before use, the stainless steel cheese vats, knives, rakes, dipping pails and metal molds were treated with chlorine solution and with scalding hot water.

The starters were prepared in skim milk which had been heated in a steamer for two hours and then cooled to the incubation temperature. *Str. lactis*, a commercial culture, was incubated at 22° C. (72° F.) and the *Str. thermophilus* starter at 37° C. (98° F.).

RESULTS

Streptococcus lactis as the only starter. In the experiments on the manufacture of Brick cheese with 0.6 per cent of *Str. lactis* as the only starter, the growth and activity of the lactic streptococci were studied during the manufacture of the cheese when the curd was cooked to 106° and to 112° F. The milk was pasteurized just before use and then cooled to the setting temperature of 88° or 90° F. The results of a typical experiment in table 1 show that according to cultural counts, *Str. lactis* remained practically constant in numbers until the sixth hour after dipping. However, the direct counts indicated a gradual increase in the numbers of *Str. lactis* and there was a small decrease in the pH of the cheese between dipping and the sixth hour. Between the sixth and twelfth hour after dipping the numbers of *Str. lactis*, as determined by direct or cultural methods, increased rapidly; after the twelfth hour the numbers increased slowly and reached their maximum between the third and sixth day. The pH at one day indicated that the cheese probably would develop a sour flavor. The results also show that a cooking temperature of 112° F., in comparison with 106° F., was harmful to the later growth and activity of *Str. lactis* in the cheese. It will be noted that *Str. thermophilus* never attained appreciable numbers in the cheese.

The data in table 1 reveal the irregularities in counts that are obtained occasionally in a bacteriological study of cheese. Since bacteria in cheese exist in colonies which vary in size and distribution, two samples from a similar location in a cheese may show considerable variation in their bacterial content. In table 1, an example of an irregular count was the excessively high count of *Str. lactis* at the sixth hour in the cheese cooked to 112° F.

Streptococcus thermophilus as the only starter. In the experiments on the manufacture of Brick cheese with *Str. thermophilus* as the only starter, the experimental conditions were the same as described in the preceding section, with the exception that 0.6 per cent *Str. thermophilus* starter was used.

The results of a typical experiment in table 2 show that *Str. thermophilus* multiply rapidly in the vat, and that after dipping the numbers increased steadily until the sixth hour; between the sixth and twelfth hours the numbers increased more slowly and reached their maximum. Cooking to 112° F. favored the growth and activity of *Str. thermophilus* although it had harmed *Str. lactis*. However, the pH at one day was considerably higher with *Str. thermophilus* than with *Str. lactis* and the later development of *Str. lactis* in large numbers with further decrease in the pH of the cheese indicated that the thermophilic streptococcus did not ferment all the sugar in the cheese.

The Brick cheese manufactured from pasteurized milk, wherein either *Str. lactis* or *Str. thermophilus* was the starter and the curd was cooked to 106° or 112° F., showed definite and characteristic defects. The cheese made with *Str. lactis* starter was sour, short and crumbly. That made with *Str. thermophilus* had a very marked fermented or fruity flavor, an excessively open texture, and a weak body. Neither of the starters produced a Brick cheese of satisfactory quality.

With different proportions of Streptococcus lactis and Streptococcus thermophilus starters. In table 3 are shown the results when 0.3 per cent each of *Str. lactis* and *Str. thermophilus* starters were used in the same cheese and two lots of cheese were made, one from raw and the other from pasteurized milk. In table 4 are recorded the results when different proportions of the two starters were used in raw milk; 0.05 per cent of *Str. lactis* and 0.5 per cent of *Str. thermophilus* starters in one lot of cheese, and 0.5 per cent of *Str. lactis* and 0.05 per cent of *Str. thermophilus* starters in the second lot. The cooking temperatures for all the cheese was 106° F.

When equal proportions of the starters were used (table 3), *Str. thermophilus* increased rapidly in numbers in the vat and reached its maximum at the third hour after dipping. At this time, *Str. lactis* was just beginning to multiply; it increased rapidly and reached its maximum numbers at the twelfth hour after dipping. The growth of the starter organisms was slower

TABLE 1

The changes in pH and bacterial content of Brick cheese during manufacture when pasteurized milk and 0.6 per cent *Streptococcus lactis* starter were used, and the curd was cooked either to 106° F. or 112° F.

Time of sampling	Cooking temperature 106° F.						Cooking temperature 112° F.					
	Millions of bacteria per gram cheese			Acidity			Millions of bacteria per gram cheese			Acidity		
	<i>Str. lactis</i>	<i>Str. thermophilus</i>	Direct counts	clumps	cells	pH	<i>Str. lactis</i>	<i>Str. thermophilus</i>	Direct counts	clumps	cells	pH
days: hours												
Milk	0.00025†	0.00025
Milk + starter	11.5	0.00025	0.13	0.13	0.13	9.10	11.0	25.8
Curd + whey	1.0	0.0002	85.0	93.0	147.0
* 0: 0	15.0	0.0005	93.0	278.0	222.0	6.38	12.0	62.0	84.0	6.34
0: 6	15.0	0.20	278.0	460.0	460.0	6.12†	390.0	0.03	0.03	710.0	1103.0	6.20†
0: 12	420.0	0.028	1234.0	1982.0	1982.0	5.49§	125.0	0.04	0.04	542.0	1041.0	5.45§
1: 0	395.0	0.17	500.0	682.0	682.0	5.08	195.0	0.21	0.21	268.0	461.0	5.07
2: 0	820.0	1.2	430.0	0.17	0.17	575.0	926.0
3: 0	2361.0	3386.0	440.0	0.034	0.034	971.0	1487.0
6: 0	1110.0	0.09	1417.0	2358.0	2358.0	180.0	0.026	0.026	553.0	1019.0
22: 0	300.0	0.0065	890.0	1257.0	1257.0	125.0	349.0	494.0
37: 0	25.0	0.005	272.0	350.0	350.0	13.5	0.20	0.20	69.0	93.0

* Zero time taken at dipping.

† . . . indicates absence of colonies in lowest dilutions.

‡ pH at one hour.

§ pH at six hours.

TABLE 2

The changes in pH and bacterial content of Brick cheese during manufacture, when pasteurized milk and 0.6 per cent *Streptococcus thermophilus* were used, and the curd was cooked either to 106° F. or 112° F.

Time of sampling	Cooking temperature 106° F.				Cooking temperature 112° F.			
	Millions of bacteria per gram cheese				Millions of bacteria per gram cheese			
	<i>Str.</i> <i>lactis</i>	<i>Str.</i> <i>thermo-</i> <i>philus</i>	Direct counts	Acidity	<i>Str.</i> <i>lactis</i>	<i>Str.</i> <i>thermo-</i> <i>philus</i>	Direct counts	Acidity
<i>days: hours</i>			<i>clumps</i>	<i>pH</i>			<i>clumps</i>	<i>pH</i>
Milk	0.0001	0.9	0.34	0.41
Milk + starter	0.53	1.5
0: 0	0.002	185.0	647.0	1059.0	2.7	3.6	9.36
0: 6	*	370.0	2134.0	4435.0	240.0	1059.0	6.32
0: 12	*	490.0	2617.0	4421.4	696.0	5.62
1: 0	0.025	620.0	873.0	1371.0	565.0	1493.0	5.62
2: 0	0.8*	590.0	902.0	1546.0	660.0	1471.0	5.37
3: 0	41.0	465.0	592.0	835.0	1000.0	1639.0
6: 0	160.0	425.0	1167.0	1688.0	660.0	1234.0	2190.0
17: 0	130.0	26.0	1446.0	1870.0	23.0	240.0	848.0	2044.0
27: 0	37.0	1.7	1597.0	2095.0	80.0	18.0	1095.0	2508.0
					175.0	15.0	1367.0	1916.0

* Tubes were so badly blown that counting was impossible.

TABLE 3

Changes in pH and bacterial content of Brick cheese during manufacture from raw and pasteurized milk, when *Streptococcus lactis* and *Streptococcus thermophilus* starters were used in a 1:1 ratio (0.3 per cent of each)

Time of sampling	Raw milk						Pasteurized milk					
	Millions of bacteria per gram cheese			Acidity			Millions of bacteria per gram cheese			Acidity		
	<i>Str. lactis</i>	<i>Str. thermophilus</i>	Direct counts	clumps	cells	pH	<i>Str. lactis</i>	<i>Str. thermophilus</i>	Direct counts	clumps	cells	pH
<i>days: hours</i>												
Milk	0.002	0.0001					0.00005	0.00005				
Milk + starter	2.9	2.2		1.7	3.8	6.35	2.20	1.4		3.8	5.9	6.35
0: 0	23.0	39.0	133.0	605.0	202.0	5.37	35.5	102.0	178.0	178.0	274.0	5.58
0: 3	115.0	675.0	605.0	1207.0	2340.0	5.30	17.0	580.0	512.0	512.0	933.0	5.49
0: 6	215.0	590.0	1207.0	757.0	1716.0	4.97	42.0	540.0	644.0	644.0	1033.0	5.25
0: 12	475.0	850.0	1716.0	517.0	708.0	5.06	130.0	690.0	1034.0	1034.0	1555.0	4.98
1: 0	340.0	690.0				5.03	240.0	795.0	900.0	900.0	1565.0	5.07
2: 0	390.0	670.0				5.06	330.0	785.0				
5: 0	200.0	75.0				5.06	170.0	155.0				
10: 0	235.0	80.0				5.06	120.0	135.0				
30: 0	46.5	2.25				5.06	19.0	1.5				

TABLE 4

The changes in pH and bacterial content of Brick cheese during manufacture when raw milk and different ratios of *Streptococcus lactis* to *Streptococcus thermophilus* were used and the curd was cooked to 106° F.

Ratio of <i>Streptococcus lactis</i> to <i>Streptococcus thermophilus</i>													
1 (0.05 per cent) : 10 (0.5 per cent)													
10 (0.5 per cent) : 1 (0.05 per cent)													
Time of sampling	Millions of bacteria per gram cheese						Millions of bacteria per gram cheese						
	<i>Str. lactis</i>		<i>Str. thermo-philus</i>		Direct counts	Acidity	<i>Str. lactis</i>		<i>Str. thermo-philus</i>		Direct counts	Acidity	
						pH						pH	
days: hours					clumps	cells					clumps	cells	pH
Milk	0.000017						0.00005						
Milk + starter	0.15		1.3		1.2	4.8	1.6		0.17		0.95	3.5	
0: 0	6.0		72.0		48.0	90.0	135.0		3.60		61.0	96.0	6.37
0: 3	5.85		205.0		435.0	685.0	250.0		270.0		623.0	970.0	5.69
0: 6	54.0		270.0		950.0	1645.0	450.0		265.0		805.0	1392.0	5.31
0: 12	68.0		340.0		760.0	1160.0	590.0		370.0		827.0	900.0	5.04
1: 0	350.0		500.0		785.0	980.0	640.0		430.0		463.0	630.0	4.93
3: 0	310.0		96.0				590.0		350.0				4.97
5: 0	230.0		275.0				530.0		140.0				4.93
11: 0	110.0		38.0				245.0		50.0				4.90
30: 0	25.0		0.3				60.0		0.25				4.97

in the cheese made from the pasteurized milk than in the cheese made from the raw milk. The most rapid drop in pH was during the first three hours after dipping, when *Str. thermophilus* was most active.

Table 4 shows that, regardless of the initial numbers of *Str. thermophilus*, its growth and activity practically ceased by the third hour after dipping. When the initial numbers of *Str. lactis* were high, growth and changes in acidity were more rapid than when the original numbers were low. Because the cheese manufactured with the larger amount of *Str. lactis* contained more moisture and hence more lactose, the final pH was lower than in the cheese made with the smaller amount of the lactic starter.

The quality of the cured cheese was recorded in accordance with the proposed system of Hanson (7), as modified by Langhus (11); that is, the flavor, texture, body and color were scored on a basis of 1 as perfect and 6 as unsalable. A perfect total score would be 4. The results in table 5 show that the cheese made from the pasteurized milk and equal parts of the starters and the cheese made from raw milk and the larger amount of *Str. thermophilus* and smaller amount of *Str. lactis* starters was of better quality than the other two lots of cheese, one of which was manufactured from raw milk and equal parts of the starters, and the other from raw milk and the smaller amount of *Str. thermophilus* and larger amount of *Str. lactis* starters. This fact demonstrated that there was a very delicate balance between the moisture content of the cheese and certain defects. A moisture content of more than 42 per cent after salting was a practical guarantee of a cheese with the defects in flavor and body characteristic of an acid cheese.

TABLE 5

A comparison of the effect of different proportions of Streptococcus lactis and Streptococcus thermophilus upon the moisture, acidity, and quality of Brick cheese

Ratio of <i>Str. lactis</i> to <i>Str. thermophilus</i>	After salting		After curing*				
	Moisture	Acidity	Score†				
			Flavor	Body	Texture	Color	Total
	<i>per cent</i>	<i>pH</i>					
1: 1 (raw milk)	44.8	4.91	3.3	3.1	3.0	1.9	11.3
1: 1 (past. milk)...	42.6	5.04	2.8	2.8	2.6	1.0	9.2
1: 10 (raw milk)	42.4	5.00	2.8	2.5	2.8	1.0	9.1
10: 1 (raw milk)	45.5	4.90	3.5	3.0	2.3	3.0	11.8

* Ages of the cheese varied from five to eight weeks.

† Scored according to system of Hanson (7) and Langhus (11), in which a value of 1 is perfect and 6 means unsalable.

DISCUSSION

When the conventional method of manufacture of Brick cheese was used and *Str. lactis* was the only starter, the cheese developed a sour flavor and

crumbly body unless special care was taken to limit the moisture at dipping. In order to produce a Brick cheese with a sweet flavor and a smooth body under the above manufacturing conditions, it was necessary to increase the length of the cooking period, and thereby decrease the moisture in the curd at dipping. Another method of controlling the moisture in the curd was to raise the cooking temperature, but it could not be increased above 106° F. or the later development of the *Str. lactis* organisms would be retarded. It was necessary to control the moisture before dipping, because during most of the draining the growth and activity of the lactic streptococci were not very extensive and their effect upon the amount of draining was negligible. Therefore, if the curd was not relatively dry at dipping, the moisture of the cheese remained high and the defects of an acid cheese developed.

When *Str. thermophilus* starter was used alone under the above manufacturing conditions, usually the cheese developed an undesirable fermented or fruity flavor and a very open texture. At the third or fourth hour after dipping, the growth and activity of the thermophilic streptococcus practically stopped, because of the unfavorably low temperature in the cheese. At that time, considerable amounts of lactose remained in the cheese and the pH was relatively high, and therefore conditions were favorable for the growth of other bacteria. Under the conditions of this investigation, the other bacteria were apparently the kinds which could ferment the lactose and lactates with the production of volatile compounds which were responsible for the fermented or fruity flavor and the gassy or open texture of the cheese.

When the proper proportions of *Str. lactis* and *Str. thermophilus* were used under the above manufacturing conditions, the defects which resulted from the use of either of the above starters alone were eliminated, and a Brick cheese of acceptable quality was produced. The reason that a combination of starters was better than either alone was that the *Str. thermophilus* organisms multiplied rapidly in the vat and during the first three hours after dipping. During this time, therefore, there was a steady production of acid and the draining was more extensive than when the thermophilic streptococcus was absent. It was pointed out in a previous statement that when *Str. thermophilus* was the only starter there would be certain defects which resulted from an incomplete fermentation of the sugar and the consequent growth of undesirable bacteria. However, in a combination of the starters, the *Str. lactis* organisms started to grow at the third hour after dipping and completed the fermentation which had been initiated by the *Str. thermophilus* bacteria. However, if the *Str. lactis* starter was used alone, the draining was more than half completed before the growth of the *Str. lactis* organisms became rapid, and the cheese retained a relatively high amount of moisture and became an acid product with characteristic defects.

The results of this investigation have indicated that in order to produce

a Brick cheese of acceptable quality by the conventional method of manufacture it was necessary to decrease the moisture of the cheese well below the legal limit of 43 per cent, otherwise a cheese with a sour flavor and a weak or crumbly body developed. A lower moisture would result in a corresponding loss in yield and hence in profit. Any method which would permit the retention of moisture up to the legal limit without harm to the quality of the product should be very desirable to the manufacturers of Brick cheese.

SUMMARY

A study was made of some of the bacteriological, chemical and physical changes in Brick cheese during its manufacture. The following results were obtained:

1. When Brick cheese was manufactured by the conventional method from raw or pasteurized milk:

a. With *Str. lactis* as the only starter special care was required in the control of the moisture at dipping, otherwise a sour flavor and a weak or crumbly body developed. If the moisture content at two days was higher than 42 per cent, the above defects developed in the cheese.

b. With *Str. thermophilus* as the only starter a fermented flavor and open texture developed in the cheese.

c. With a combination of the above two starters, a better quality of cheese was produced than when either was used alone.

2. During the manufacture of Brick cheese, the growth and activity of the starter bacteria were as follows:

a. *Str. thermophilus* practically stopped growth at the third or fourth hour after dipping because of the relatively low temperature in the cheese.

b. The growth and activity of *Str. lactis* were not very extensive until the latter part of the draining period. It usually reached its maximum numbers within one or two days. When the original inoculum was large (0.5 per cent or more) growth and activity were more extensive during the early part of the draining period.

3. The cooking temperature could not be raised above 106° F. without greatly retarding the growth and activity of *Str. lactis*. However, a cooking temperature of 112° F. increased the rate of growth and activity of *Str. thermophilus*.

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THE EFFECT OF HYDROGENATION ON THE NUTRITIVE VALUE OF THE FATTY ACID FRACTIONS OF BUTTER FAT AND OF CERTAIN VEGETABLE OILS*

R. K. BOUTWELL, R. P. GEYER, C. A. ELVEHJEM AND E. B. HART

Department of Biochemistry, University of Wisconsin, Madison, Wisconsin

Studies on the comparative nutritive value of butter fat and certain vegetable oils (1) showed that butter fat homogenized into raw skimmed milk with ample fat soluble vitamins and minerals added and fed *ad libitum* to weanling rats gave better growth than did corn oil, coconut oil, cottonseed oil, and soybean oil fed in a like manner. Further study disclosed that the factor (or factors) responsible for the superior growth of rats on butter fat milk did not lie in the non-saponifiable fraction of butter fat (1), nor did such compounds as egg lecithin, sphingomyelin, sphingosin, or ethanol amine have any effect on the nutritive value of the vegetable oils used in our experiments. Choline was also tried and showed slight growth promoting effects on the females (2). Later experiments (unpublished) have shown that the addition of choline (30 mg. per day) has had no growth promoting effect when added to butterfat or any of the vegetable oils fed in skimmed milk. The separation of the fatty acids of butter fat into a volatile fraction by steam distillation and into a saturated and an unsaturated fraction by lead soap solubilities in alcohol was carried out. Feeding the glycerol esters of each of these fractions in corn oil indicated that the factor responsible for the superior growth with butter fat milks lay in the saturated fraction of butter fat (3).

To gain further insight as to the nature of the substance responsible for the better growth promoting value of butter fat milk, complete catalytic hydrogenation of the saturated fraction was necessary to eliminate the possibility that an unsaturated compound insoluble as the lead soap or carried along with the insoluble lead soaps was the active principle of the saturated fraction. The iodine numbers of various preparations of the saturated fraction varied from 6 to 10. The effect of hydrogenation on the nutritive value of the unsaturated fraction was also determined in the present study. In view of the increased growth obtained on the hydrogenated butter fat fractions, feeding experiments were also carried out on various hydrogenated vegetable oils. The results of this experiment are included in this paper.

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EXPERIMENTAL

The fatty acid fractions of butter fat were prepared essentially by the method of Hilditch (4) and as previously reported by us (3). The steam volatile fraction was discarded. The triglycerides of the saturated and the unsaturated fractions were prepared by heating theoretical amounts of the fatty acid fractions and glycerol together at 200° C. in a three-neck round-bottom flask for 6 hours with an efficient mercury seal stirrer and in an atmosphere of carbon dioxide (5). Both the saturated and unsaturated glycerides were completely hydrogenated over Raney nickel at 150° C. and 1600 to 2200 pounds pressure without a solvent.¹

All feeding trials were carried out in the usual manner (1, 2, 3). Weanling rats about 20 days old were used throughout. Three males and three females in individual cages were placed in each group. The fats were homogenized into fresh raw skimmed milk at a 4 per cent level with a laboratory homogenizer. Ten micrograms of carotene were dissolved in each gram of butter fat and 20 micrograms of carotene were added to each gram of the other fats. All rats were irradiated 10 minutes each day and 100 micrograms of α -tocopherol acetate were given to each rat every week. The milk was mineralized so that every 100 cc. contained 1.5 mg. of iron and 0.15 mg. each of copper and manganese. The milks were fed *ad libitum* and consumption records kept, care being taken to feed each rat only a small excess over the amount that it could consume in one feeding.

Six groups of rats—three males and three females in each group for each experiment—were set up and fed as follows for 6 weeks:

Group I—Butter fat

“ II—Corn oil

“ III—Corn oil plus saturated fraction butter fat

“ IV—Corn oil plus hydrogenated saturated fraction butter fat

“ V—Corn oil plus unsaturated fraction butter fat

“ VI—Corn oil plus hydrogenated unsaturated fraction butter fat

The glycerides of each fraction were mixed in *equal proportions* with corn oil (Mazola) each day just before homogenization into the skimmed milk. As above stated the total fat content of the milk was 4 per cent. Three such experiments were carried out.

Growth of the rats on the hydrogenated butter fat fractions in grams during the first three weeks of the experiment is tabulated in table 1. The average grams gained as shown in the last line, representing 9 males and 9 females in each group (except groups III and V where 6 males and 6 females are represented) are graphed in figure 1. The animals on corn oil plus the saturated fraction of butter fat grew slightly faster than animals on butter fat and considerably faster than animals on corn oil, while the

¹ We wish to thank Dr. Homer Adkins of the University of Wisconsin Chemistry Department for the hydrogenation of the fats.

TABLE 1

Growth on the hydrogenated fatty acid fractions

The figures show grams gain made during the first three weeks on the experiment. Each figure represents the average of three rats.

Diet	Butter fat		Corn oil		Corn oil plus sat. fract.		Corn oil plus hyd. sat. fract.		Corn oil plus unsat. fract.		Corn oil plus hyd. unsat. fract.	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
Expt. 22	70	62	67	58	80	69	69	73	63	63	81	75
Expt. 24	87	71	64	57	72	68	88	70
Expt. 29	72	67	60	60	80	69	76	71	73	53	90	71
Average	76	67	64	58	80	69	72	71	68	58	86	72

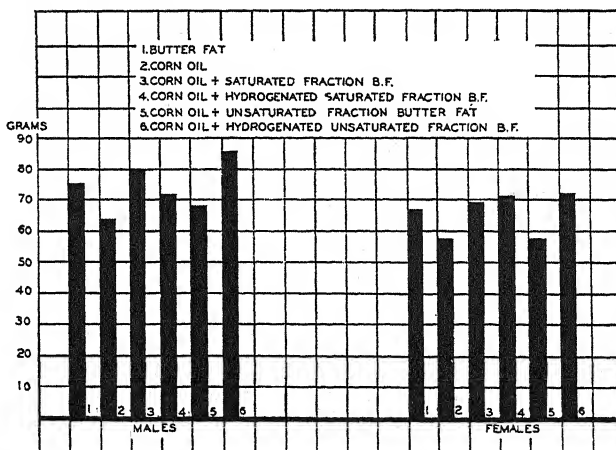


FIG. 1. Average gains made during the first three weeks on experiment by male and female rats representing 18 rats (9 males and 9 females) for each bar, except bars 3 and 5 where 6 males and 6 females are represented.

animals on corn oil plus the unsaturated fraction grew at about the same rate as those on corn oil. This confirms our earlier findings (3). Rats fed the hydrogenated saturated fraction grew at about the same rate in the case of the females and somewhat slower in the case of the males than those on the saturated fraction. Rats on corn oil plus the hydrogenated unsaturated fraction grew much better than those of any other group. The fur coat of the rats on the saturated and hydrogenated butter fat fractions was superior to that of the rats on corn oil.

In table 2 are recorded the cc. of milk consumed for each gram of gain in weight. In the case of the butter fat only and the various butter fat fractions, the amounts of milk ingested are quite constant. Somewhat higher amounts of skimmed milk, reinforced with corn oil only, were re-

TABLE 2

Number of cc. of milk required to produce one gram gain in weight over the first three weeks on experiment

Diet	Butter fat		Corn oil		Corn oil plus sat. fract.		Corn oil plus hyd. sat. fract.		Corn oil plus unsat. fract.		Corn oil plus hyd. unsat. fract.	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
Expt. 22	12.2	13.1	13.5	13.8	13.5	13.6	13.3	14.4	13.3	13.5	13.0	14.3
Expt. 24	13.1	13.6	14.3	16.1	12.8	13.4	11.2	13.8
Expt. 29	11.8	13.1	13.0	13.2	11.7	13.5	12.2	13.4	11.2	14.7	11.7	12.9
Average	12.3	13.3	13.6	14.4	12.6	13.6	12.8	13.7	12.3	14.1	12.0	13.3

quired for each gram of gain, although the differences may not be especially significant.

For study of the effect of hydrogenation on their nutritive properties, corn oil, coconut oil, cottonseed oil, and soybean oil were completely hydrogenated over Raney nickle. These oils were refined products, with the exception of soybean oil, which was crude. The hydrogenated corn oil, hydrogenated cottonseed oil, and hydrogenated soybean oil were each mixed 1 part to 3 parts of the same oil not hydrogenated. The hydrogenated coconut oil was mixed in equal proportions with unhydrogenated coconut oil. A commercial, partially hydrogenated cottonseed oil (a shortening) was also fed in this experiment. Ten groups were set up, each group consisting of three males and three females. They were fed skimmed milk in the usual manner, with mineral and fat soluble vitamin fortification.

Group	Component fats	Per cent of total fat	Total fat per cent
1	Butter fat	100	4
2	Corn oil	100	4
3	Hydrogenated corn oil	33	4
	Corn oil	66	
4	Coconut oil	100	4
5	Hydrogenated coconut oil	50	4
	Coconut oil	50	
6	Cottonseed oil	100	4
7	Hydrogenated cottonseed oil	33	4
	Cottonseed oil	66	
8	Soybean oil	100	4
9	Hydrogenated soybean oil	33	4
	Soybean oil	66	
10	Crisco	100	4

Table 3 shows the average gains in grams of the males and females in each group at the end of 3 weeks and at the end of 6 weeks. The gains at the end of 3 weeks are graphed in figure 2. In all cases hydrogenation of the vege-

TABLE 3

Growth on the hydrogenated vegetable oils showing average grams gained during the first three weeks and total grams gained (each figure represents three rats)

Diet	Butter fat		Corn oil		Corn oil plus hyd. corn oil		Cocunut oil		Cocunut oil plus hyd. cocunut oil		Cotton-seed oil		Cottonseed oil plus hyd. cotton-seed oil		Soybean oil		Soybean oil plus hyd. soybean oil		Grisco
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	
At 3 wks. (grams)	73	64	62	57	57	53	47	47	42	42	72	59	50	55	73	61	51	53	62 53
At 6 wks. (grams)	141	128	131	111	119	98	88	90	107	89	157	113	110	107	159	113	107	98	131 106

TABLE 4

Growth on the hydrogenated vegetable oils showing number of cc. of milk required to produce one gram gain in weight over the first three weeks on experiment (6 animals on each fat—3 males, 3 females)

Diet	Butter fat		Corn oil		Corn oil plus hyd. corn oil		Cocunut oil		Cocunut oil plus hyd. cocunut oil		Cotton-seed oil		Cottonseed oil plus hyd. cotton-seed oil		Soybean oil		Soybean oil plus hyd. soybean oil		Grisco
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	
Sex																			
cc.	11.9	14.0	12.7	14.1	13.8	15.4	14.4	16.8	16.0	17.4	12.1	13.9	14.1	14.6	12.5	13.7	15.4	16.8	12.8 15.8

table oils did not improve their growth promoting properties. These results show that certain male rats on cottonseed oil and soybean oil gained about the same amount in the first 3 weeks as those on butter fat and at the end of 6 weeks had gained somewhat more than the males on butter fat. The partially hydrogenated cottonseed oil gave about the same growth as corn oil and was slightly better than the hydrogenated cottonseed oil—cottonseed oil mixture in the case of the males. In an earlier trial corn oil was mixed in equal parts with completely hydrogenated corn oil and fed with results similar to those secured in this experiment.

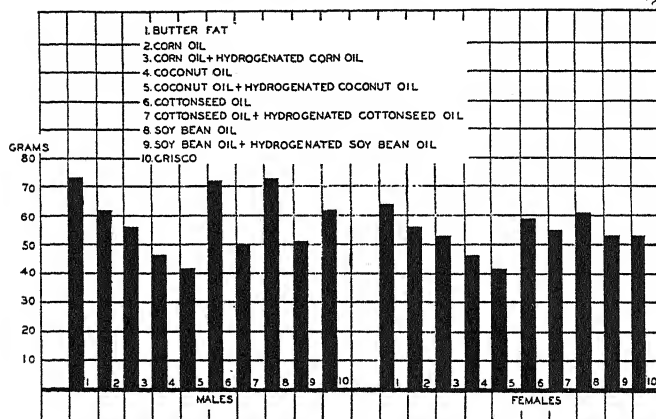


FIG. 2. Average gains made during the first three weeks on experiment of male and female rats representing 6 rats (3 males and 3 females) for each bar.

Records of milk consumption per gram of weight increase are recorded in table 4. The butter fat fed rats show a slightly lower milk consumption per gram of growth, particularly the males. In the case of the females there was a slightly greater efficiency in the case of cottonseed oil and soybean oil than with butter fat. In any case the differences are too small to be of significance. As already stated, earlier published data as well as unpublished data show generally that butter fat is superior to cottonseed oil and soybean oil in *ad libitum* feeding with skimmed milk as the basal diet. An occasional rat, through synthetic ability or storage will grow quite as well on some of the vegetable oils as on butter fat, thereby giving an average result contrary to the general findings.

DISCUSSION

The results obtained on the hydrogenated butter fat fractions indicate that the superiority of butter fat lies in the saturated fraction. The unsaturated fraction added to corn oil gave no response in growth over corn oil alone, indicating that the superior growth-promoting property of butter fat is probably not due to an unsaturated fatty acid. Since either fraction com-

pletely hydrogenated gave growth as good or better than butter fat itself, it is probable that a long chain saturated fatty acid (or acids) is responsible for the superior growth-promoting value of butter fat. It is evident that the unsaturated fraction of butter fat contains an unsaturated form of the compound which may readily be converted to the active form by catalytic hydrogenation. The better growth obtained with certain fractions over the butter fat alone might well be explained by the fact that in the mixtures of these fractions with corn oil there was more of the high molecular weight saturated fatty acid (or acids) present than in butter fat alone.

The unsaturated form of the growth-promoting saturated fatty acid is apparently not present in the vegetable oils studied. The poorer growth found on feeding hydrogenated vegetable oils may be a factor of digestibility, since the hydrogenated vegetable oils had high melting points. This possibility was not studied. Individual male rats on cottonseed oil and soybean oil showed somewhat better growth than the control rats on butter fat in this experiment. This was the only experiment in which this occurred and was an individual performance. Earlier experiments (1) and more recently secured data (unpublished) on cottonseed oil and soybean oil show that these oils are inferior to butter fat in growth promoting value.

In this investigation, as well as those previously recorded on the same subject, we have used the "*ad libitum*" method of feeding rather than the "paired feeding" method. We have done this because we believe that the "*ad libitum*" method does not penalize the more efficient ration and serves to emphasize any distinctive nutritive differences. The critics of *ad libitum* feeding for these types of experiments maintain that differences in growth are due to increased appetite, greater palatability and greater consumption, rather than to some needed nutrient. Of course, this is exactly the result of a superior food over an inferior food. A favorable physiological response engendered by the superior food results in a greater consumption and better growth.

Since in these experiments the only variable was the fat or fat fractions, then a fat or oil giving better results in growth than another fat or oil must accomplish this through its effect on the animal's physiology. Hardly could one expect a fat fraction, after the chemical manipulation to which it has been subjected, to improve the appetite as compared with the raw corn oil. The consumption records support no such view. Corn oil invariably induced inferior growth to that obtained with butter fat or butter fat fractions and the daily consumption was also less.

We are convinced that the *ad libitum* feeding method in this type of experimentation will more clearly express any difference in the nutritive value of the fats or oils under investigation than a method that limits consumption. We think we are correct when we say that no new dietary factor has ever been disclosed by the use of the "paired feeding" method. A con-

trol animal limited in its intake to the amount of food consumed by an animal suffering from a deficiency must sooner or later have its consumption reduced to zero. Ultimately that is exactly the consumption level reached by an animal fed a deficient ration. Only short-time records will obliterate such a condition unless internal synthesis wholly or partially intervenes.

CONCLUSIONS

1. The superior growth-promoting property of butter fat as compared to certain vegetable oils is probably due to a saturated compound; apparently a long chain saturated fatty acid (or acids) present in small amounts in butter fat is responsible for these properties of butter fat.

2. The unsaturated fraction of butter fat is relatively rich in an unsaturated form of this compound which by hydrogenation may readily be converted to the active compound.

3. Certain vegetable oils as corn oil, coconut oil, cottonseed oil and soybean oil apparently do not contain the unsaturated form of this compound. Hydrogenation of these vegetable oils did not improve their nutritive value when incorporated into skimmed milk.

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OXIDIZED FLAVOR IN MILK. X. THE EFFECT OF FEEDING
POTASSIUM IODIDE SUPPLEMENTS TO DAIRY COWS ON
THE CAROTENE CONTENT OF THE BUTTER FAT AND
ON THE ASCORBIC ACID CONTENT OF THE MILK
AND THE RELATIONSHIP TO METAL-INDUCED
OXIDIZED FLAVOR*

W. CARSON BROWN,¹ A. H. VANLANDINGHAM,² AND CHAS. E. WEAKLEY, JR.²
West Virginia Agricultural Experiment Station, Morgantown

It has been found that the level of the iodine content in milk follows the same seasonal variation as the susceptibility of milk to oxidized flavor. Likewise, the carotene content of the butter fat follows this same seasonal trend. Koch (6) first reported changes in the iodine content of the thyroid gland with the changing seasons. Later, Seidell and Fenger (15) and Fenger (4) made studies of the iodine content of the thyroids of cattle, hogs, and sheep, special attention being paid to seasonal variations. It was found that there were two or three times as much iodine present in the thyroid in the months between June and November as in the months between December and May. Kendall and Simonsen (5) likewise found seasonal variation in the thyroid of swine. Mathews, Curtis, and Meyer (8) found that during the late spring the milk iodine from both normal and iodine-supplemented cows was unusually low. The milk from the cows receiving iodine contained from 7 to 26 times as much iodine as that from the control cows. Ralston and co-workers (13) reported that the fact is well established that iodine is a normal constituent of the thyroid gland and that iodine must be supplied to an animal for normal activity of the gland which in turn reflects its importance on metabolic activity.

Treichler *et al.* (17) and Fashold and Heideman (3) found that goat butter contained vitamin A but very little carotene. The latter reported that after thyroidectomy, vitamin A *per se* is absent and large amounts of carotene are present.

Since the level of the iodine in the milk follows the same seasonal change as the susceptibility of milk to oxidized flavor, and since it has been demonstrated in the case of the goat that the thyroid affects the ratio of vitamin A to carotene in the milk, it seemed possible that iodine through its effect on the thyroid might bring about a change in the susceptibility of milk to metal-induced oxidized flavor. Accordingly the following experiment was planned and conducted.

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¹ Department of Dairy Husbandry.

² Department of Agricultural Chemistry.

EXPERIMENTAL

Five Jersey cows, whose milks were susceptible to oxidized flavor when contaminated with copper, were selected from the regular milking herd. In addition to the regular herd ration, each cow was given a supplement of 5 grams of potassium iodide daily for a period of 14 days. All other factors were maintained as nearly uniform as was possible.

Three quarts of milk were collected from each cow at the morning milking on the first three consecutive days of each week and carotene, ascorbic acid, and flavor determinations were made. The ascorbic acid was determined on the individual samples of raw milk, as soon as possible after milking, by titrating as suggested by Sharp (16). The remaining milk was pasteurized in bottles. Following pasteurization and cooling, four one-half

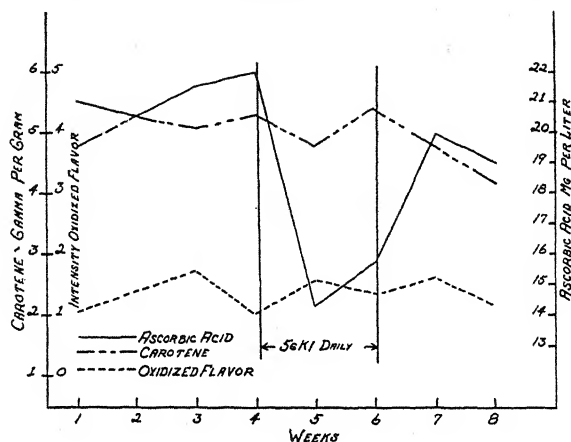


FIG. 1. The effect of potassium iodide supplements on the carotene content of the butter fat and the ascorbic acid content of the milk and the relationship to metal-induced oxidized flavor.

pint samples were prepared containing none, 0.5, 1.0, and 1.5 parts per million, respectively, of copper from a copper sulphate solution. These samples were then stored in ice water for three days, after which they were scored for flavor by at least three judges familiar with oxidized flavor. The remainder of the milk was prepared for carotene analysis by gravity separation of the cream followed by churning. Before churning, the cream from each of the three days was composited so as to make one churning and one analysis for carotene per cow per week. The butter thus obtained was melted and centrifugalized in Hart's casein tubes in an electrically heated centrifuge for 15 minutes, after which the clear, liquid butter oil was decanted into a clean, dry jar. The carotene analyses were made according to the method of Baumann and Steenbock (1) modified by Rogers and associates (14). After a four-week preliminary period on normal herd ration, each of the cows was given a daily supplement of 5 grams of

potassium iodide. The potassium iodide was administered by drenching so that the cows received the full amount of the dosage. The supplement was given for 14 days. Three of the cows upon finishing the potassium iodide feeding period and a two-week readjustment period were dropped from the experiment. Following a seven-day readjustment period the remaining two animals were given another 14-day potassium iodide feeding period. At the end of this period the experiment was discontinued. The dosage during the second feeding period was likewise 5 grams daily.

The results of this study are shown in figures 1 and 2. Figure 1 shows the results obtained with the three cows which received the supplement for only one period. These results show that there was a decided decrease in the amount of ascorbic acid secreted in the milk. As soon as the supplement was discontinued the ascorbic acid rose very sharply to approximately normal. The carotene content of the butter fat and the intensity of the metal-induced oxidized flavor remained fairly constant throughout the experiment.

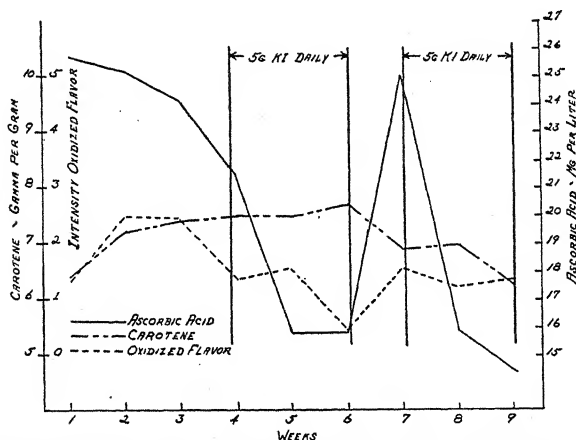


FIG. 2. The effect of potassium iodide supplements on the carotene content of the butter fat and the ascorbic acid content of the milk and the relationship to metal-induced oxidized flavor.

Figure 2 shows the same general type of results as figure 1 except that the ascorbic acid content was lowered a second time by the feeding of potassium iodide. This shows clearly that the reduction in ascorbic acid in the first three animals was not coincidental. Here also no effect was noted in the level of the carotene or in the intensity of the oxidized flavor.

DISCUSSION

The feeding of 5 grams daily of potassium iodide was sufficient to reduce the ascorbic acid content of the milk approximately one-third. The potassium iodide supplement, however, did not affect the carotene content of the butter fat or the intensity of metal-induced oxidized flavor. This

indicates that the ascorbic acid content of the milk may not be an important factor in the development of oxidized flavor and supports recent information as reported by the authors (2).

There may be a very different and indirect application of these results. Phillips and Stare (12) found a high concentration of ascorbic acid in the pituitary gland of cattle and later Phillips and co-workers (11) reported a low blood plasma ascorbic acid content in cattle fed on restricted dietary regimes. Likewise, it was shown by Lardy and Phillips (7) that fresh semen from bulls with low fertility had less than 2 mg. of ascorbic acid per 100 cc., and in some cases only a trace was found. Good breeding bulls, on the other hand, produced semen containing 3.0–8.0 mg. of ascorbic acid per 100 cc. of fresh semen. In a later work Phillips *et al.* (10) showed that the subcutaneous injection of ascorbic acid results in the restoration of the fertility of certain bulls. During the past year these workers (9) have shown that ascorbic acid is intimately associated with the early phases of the reproductive processes and that it can be successfully used as a therapeutic measure in treating certain types of sterility in the cow. From these findings it seems possible that heavy iodine feeding might affect the ascorbic acid level of the blood and thus influence breeding efficiency. It is a common belief among some veterinarians and animal breeders that heavy iodine feeding rendered cows sterile for a period following such treatment, but a review of the literature thus far has failed to reveal experimental evidence on this point. In view of the effect of potassium iodide on the ascorbic acid content of the milk it seems entirely possible that potassium iodide might lower breeding efficiency by affecting the ascorbic acid content of the blood. However, further experimental proof is needed to substantiate or disprove the effect of potassium iodide on the efficiency of breeding.

SUMMARY AND CONCLUSIONS

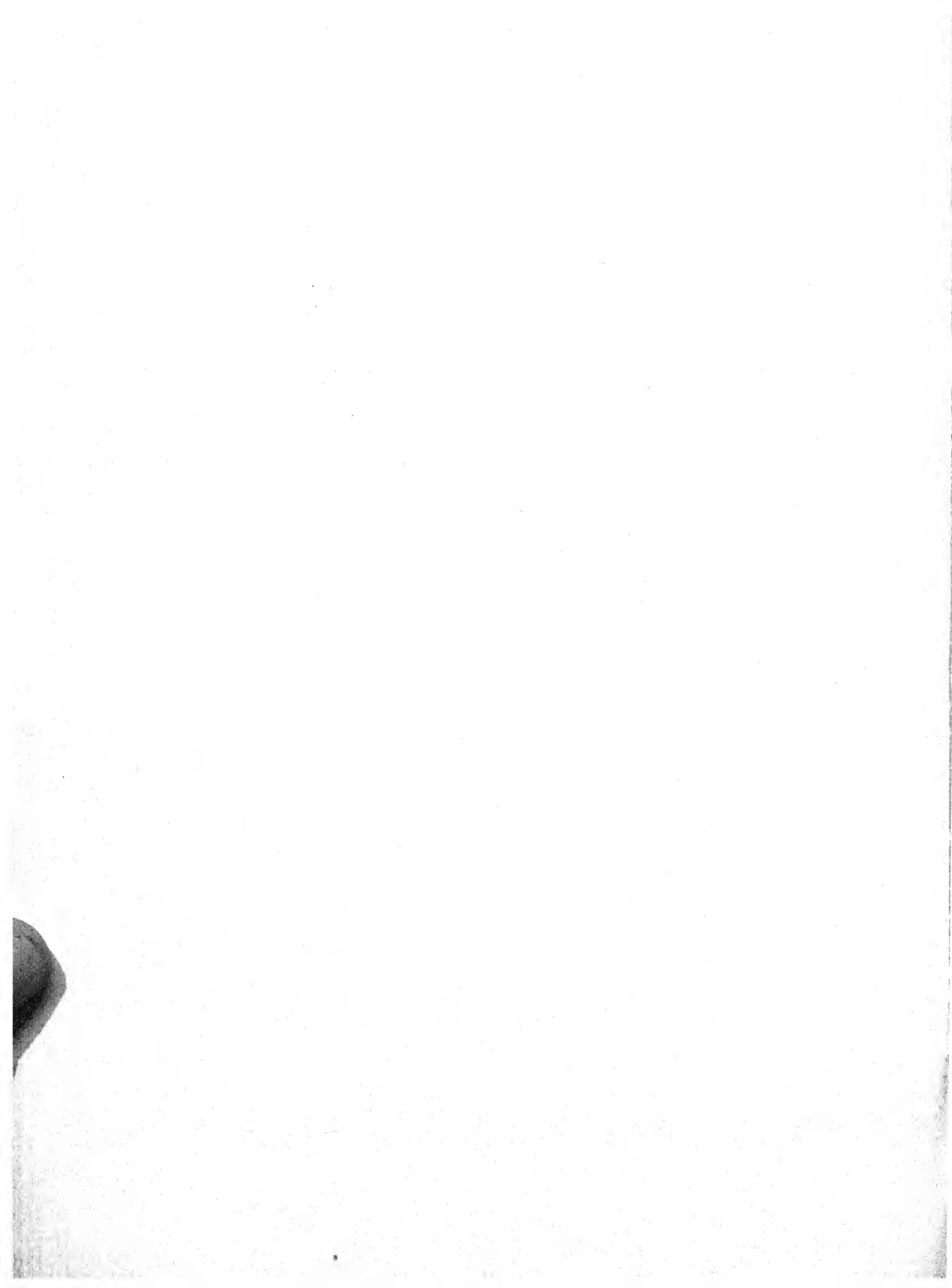
1. The feeding of 5 grams daily of potassium iodide for fourteen days lowered to a marked degree the percentage of ascorbic acid secreted in the milk, but had no noticeable effect on the level of the carotene content of the milk.
2. The decrease in the ascorbic acid content of the milk did not produce a corresponding increase in the intensity of the metal-induced oxidized flavor.
3. From these results it appears that the level of the ascorbic acid in the milk may not be as great a factor in the production of milk with low susceptibility to oxidized flavor as was formerly believed.

ACKNOWLEDGMENT

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AN ANALYSIS OF THE RELATIONSHIP BETWEEN THE CURD TENSION AND THE CURD SURFACE AREA OF MILK

ARNOLD B. STORRS

American Seal-Kap Corporation, Long Island City, N. Y.

For the past several years there has been a growing interest within the dairy industry in the commercial preparation of so-called modified milk, or fluid milk with improved digestibility. This has naturally led to a more intensive study on the part of research workers of the factors related to digestibility. Many attempts have been made to develop new and possibly more accurate *in vitro* tests which would simplify control problems. The curd tension test, originally proposed by Hill (1) and subsequently modified by others (2, 3), is still the only generally accepted test of this nature and its wide use has, in fact, led to the general designation of such modified milks as "soft curd milk."

In several of the techniques which have been proposed recently (4, 5, 6, 7, 8) the property of curd particle size or curd surface area has had an important bearing upon the estimation of comparative digestibility. These studies have led many to agree with the viewpoint expressed by Doan (9), that the size of the curd particles obtained in peptic coagulation under conditions of agitation and acidity closely approximating those existing in the stomachs of young infants would be a more suitable index of digestibility than the toughness of the curd formed without any agitation.

Since the curd tension test and the newer methods which have been suggested are all intended as *in vitro* measures of digestibility, the question of correlation between the results obtained by different procedures naturally arises. Chambers and Wolman (6) reported some agreement between curd tension and curd surface area. Their conclusions were based upon a comparison of different types of milk and the determinations of curd surface area were made at pH levels of about 4.5 to 5.0 only. They also observed that there were some apparent exceptions.

It was the purpose of this investigation to make a statistical analysis of the relationship between curd tension and curd surface area as it exists both within individual types of milk and within groups of mixed samples of different milks.

EXPERIMENTAL

Source of samples: Several types of milk were tested, including untreated milk, homogenized milk (high pressure), enzyme-treated milk (pancreatic enzyme) and base exchange milk. All of the milk analyzed was purchased from milk plants that were making or selling the particular type of modified milk.

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Curd tension: The technique tentatively recommended by the Curd Tension Committee of the American Dairy Science Association (10) was followed. A Submarine Signal curdometer was employed for making the readings and a thermostatically controlled water bath maintained at $95 \pm 1^\circ$ F. was used for tempering the reagents and the samples and for incubating the test samples.

Curd surface area: Curd surface area was determined by the Chambers-Wolman test (5, 6) using the modified technique described by Anderson (8). Briefly, 100 ml. of milk was coagulated in thin-walled latex sacs by the addition of sufficient pepsin-hydrochloric acid coagulant (equal parts of a 0.6 per cent aqueous solution of pepsin, U.S.P. 1:3000, and Normal HCl) to give the desired pH. After a "digestion" period of thirty minutes with constant mechanical agitation the samples were emptied into individual containers and hardened with formaldehyde. Curd surface area values were calculated after washing the curds through a series of graduated sieves and weighing the various amounts of each size fraction. Determinations were made at pH levels of 6.0, 5.5, 5.0, 4.5 and 4.0.

pH values: Measurements of pH were made at 25° C., using a Beckman pH meter equipped with remote electrodes.

Statistical analysis: Calculation of the coefficients of correlation (r) and determinations of the significance of the data were made according to the method outlined by Paterson (11). In determining significance the "5 per cent" point was used. In other words, a coefficient of correlation was judged to be significant only when the data indicated that a similar degree of correlation could probably have been obtained purely as a matter of chance in less than 5 per cent of the trials.

RESULTS

The values for curd tension and curd surface area of the untreated, homogenized, enzyme-treated and base exchange milks are shown in table 1.

TABLE 1

The average values for curd tension and curd surface area (S/gm.) of all samples tested

Type of milk	No. of samples	Curd tension	Chambers-Wolman test				
			pH 6.0	pH 5.5	pH 5.0	pH 4.5	pH 4.0
			gms. S/gm.	S/gm.	S/gm.	S/gm.	S/gm.
Untreated	11	37.7	5.8	6.3	10.7	27.5	50.9
Homogenized	10	18.0	6.3	6.9	15.0	39.4	52.3
Enzyme-treated	11	17.8	5.6	6.4	17.5	49.0	71.8
Base exchange	10	6.7	35.9	9.5	11.3	18.3	44.5

In table 2 are given the coefficients of correlation within each type of milk between the curd surface area at various pH levels and the curd tension.

TABLE 2

The correlation within types of milk between the curd surface area at various pH levels and the curd tension

Type of milk	Coefficient of correlation				
	pH of Chambers-Wolman test				
	6.0	5.5	5.0	4.5	4.0
Untreated	<i>r</i> +.03	<i>r</i> +.32	<i>r</i> -.16	<i>r</i> -.27	<i>r</i> -.18
Homogenized	+.01	+.49	-.27	-.02	-.08
Enzyme-treated	+.24	+.25	+.06	+.38	-.04
Base exchange	-.65*	-.67*	+.34	+.19	+.30

* Indicates coefficient of correlation was significant. Upon recalculation of these values after the elimination of three samples having curd tensions of 20, 14 and 17 grams the following values for *r* were obtained: pH 6.0, -.34; pH 5.5, +.14. Neither of these was significant.

A statistically significant relationship was observed in only two instances, namely in the case of base exchange milk when the determinations of curd surface area were carried out at pH 6.0 and pH 5.5. Since there was reason for suspecting that the apparent correlation in these two cases was due to the fact that at least three of the samples of base exchange milk had probably been undertreated, as indicated by the curd tension tests, these correlation coefficients were recalculated after the elimination of the samples having curd tensions of 20, 14 and 17 grams. After this procedure the correlation coefficient values dropped to -.34 and +.14 respectively, neither of which was of significance.

The relationship between curd surface area and curd tension within groups of various combinations of modified milks is shown in table 3. In calculating these values all samples of each type of milk included in the various groups were taken into account. First, all four types of milk were taken together and the coefficients of correlation calculated for the group as a whole. Then all possible combinations of three different types of milk were calculated. Considering all the various groups of milk, the relationships ranged from a significant negative correlation to a significant positive correlation with a good number of the values indicating no significant relationship.

There was no significant relationship between curd tension and curd surface area within the following types of milk: untreated, homogenized, enzyme-treated and base exchange milk. Under different conditions the correlation coefficients of these milks varied considerably, showing both positive and negative correlation, but in no instance were the values high enough to indicate that they might not have been arrived at through pure chance. The apparently significant relationship observed at first in base exchange milk (table 2) was evidently due to the presence of some un-

TABLE 3

The correlation within groups of various combinations of modified milks between the curd surface area at different pH levels and the curd tension

Types of milk	Coefficient of correlation				
	pH of Chambers-Wolman test				
	6.0	5.5	5.0	4.5	4.0
	<i>r</i>	<i>r</i>	<i>r</i>	<i>r</i>	<i>r</i>
Untreated					
Homogenized					
Enzyme-treated	-.62*	-.51*	-.16	+.05	+.01
Base exchange					
Untreated					
Homogenized	-.02	+.08	-.61*	-.56*	-.43*
Enzyme-treated					
Base exchange					
Untreated					
Homogenized	-.71*	-.58*	-.12	+.20	+.21
Enzyme-treated					
Base exchange	-.70*	-.61*	-.14	+.01	+.01
Homogenized					
Enzyme-treated	-.72*	-.56*	+.41*	+.48*	+.38*
Base exchange					

* Indicates coefficient of correlation was statistically significant.

dertreated samples, the elimination of which resulted in a finding of no significant relationship.

With regard to the correlation between curd surface area and curd tension within mixed groups of different types of milk the data were variable. In general, at pH levels of 6.0 and 5.5 a significant relationship was observed only in those groups which included base exchange milk. Conversely, at pH levels of 5.0, 4.5 and 4.0 the only significant negative correlation was observed in the group in which no base exchange milk was included. In the last group shown in table 3, including homogenized, enzyme-treated and base exchange milk, a significant negative correlation was observed at pH 6.0 and pH 5.5 while at pH levels, of 5.0, 4.5 and 4.0 the relationship was significantly positive.

In a previous paper (12) data were presented showing the varying response of some types of milk to changes of pH in the Chambers-Wolman test for curd surface area. It was pointed out that the method by which the milk had been treated in modification was apparently one of the most important factors involved since each type had some characteristics largely peculiar to itself. In the present comparison of curd tension and curd surface area it seems probable that the lack of a consistent relationship is due to the same factor, *i.e.*, the individual characteristics peculiar to each type of milk.

It would normally be expected with respect to good digestibility in milk that low curd tension would be associated with high curd surface area, or,

in other words, that there would be an inverse or negative relationship between the results of these tests. It now appears that this is not necessarily true since the data obtained in this investigation indicate that the degree of correlation depends to a very great extent upon the types of milk included in the tests. It would be possible by selective sampling to attain almost any desired degree of relationship.

In view of these findings it seems likely that such correlation as has been observed between curd tension and curd surface area has been largely coincidental. It is probable that curd tension and curd surface area are independent characteristics of milk and that each may be influenced or determined by different or loosely related factors.

SUMMARY

Within individual types of commercially modified milks there appears to be no significant correlation between curd tension and curd surface area.

Within mixed groups of various types of modified milk the relationship between curd tension and curd surface area is of variable significance and seems to depend upon the types of milk included in the particular group.

It appears that curd tension and curd surface area are independent characteristics of milk and that each may be influenced or determined by factors not closely related.

ACKNOWLEDGEMENT

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A METHOD FOR THE ESTIMATION OF NICOTINIC ACID IN MILK¹

E. A. BAILEY, JR.,² W. J. DANN, G. HOWARD SATTERFIELD

AND

C. D. GRINNELLS³

Since the discovery of the vitamin activity of nicotinic acid by Elvehjem *et al.* in 1937 (3), much attention has been given to the development of a chemical method for its estimation in biological materials. The König reaction (10) in which the pyridine nucleus, with cyanogen bromide and an aromatic amine, yields a substance which can be estimated colorimetrically, has been made the basis of the most satisfactory methods of estimation. In the application of the different methods to biological materials, it has been found difficult to obtain nicotinic acid extracts which are free from interfering materials. Perlzweig, Levy, and Sarett (13) have developed a method for determination of nicotinic acid in urine by which an almost colorless extract is obtained and this difficulty largely overcome. Dann and Handler (2) have adapted this method for estimation of nicotinic acid to animal tissue. The present study deals with the adaptation of the method to the estimation of nicotinic acid in milk.

The essentials of the method are: acid hydrolysis, removal of interfering substances by the use of Lloyd's reagent and lead hydroxide, and development of color by the method of Bandier and Hald (1).

METHOD

Preparation of extract: The whole milk is centrifuged for several minutes and the skim milk siphoned off. A 5-ml. sample of the skim milk is transferred to a large test tube (50 ml. capacity), graduated at 25 ml. Five ml. of concentrated hydrochloric acid (sp. gr. 1.19) are added and the tube placed in a boiling water bath for one hour. Charring and blackening occur. The tube is rotated occasionally to insure thorough mixing. This acid digestion completely hydrolyzes any nicotinamide which may be pres-

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² Submitted by E. A. Bailey, Jr., in partial fulfillment of the requirements for the degree of Master of Science, North Carolina State College of the University of North Carolina.

³ Research Fellow, North Carolina State College; Associate Professor of Physiology and Nutrition, Duke University School of Medicine; Professor of Biochemistry, North Carolina State College; Associate, in charge of Dairy Research, North Carolina Agricultural Experiment Station, respectively.

ent. At the end of this time the tube is cooled, the contents made up to the mark with distilled water, and the solid material allowed to settle. A 15-ml. aliquot of the clear, dark brown supernatant liquid is transferred to a small beaker, 2 ml. of 15 N sodium hydroxide are added, and the solution cooled and the pH adjusted to 1 using the glass electrode. The solution is transferred, with a minimum of water, to an 18 × 135 mm.-Pyrex test tube containing 2 g. of Lloyd's reagent, the tube having previously been graduated at 16.2 ml. so as to contain 15 ml. of liquid in addition to the 2 g. of Lloyd's reagent. The tube is shaken for one minute, centrifuged, and the supernatant liquid poured off and discarded. The Lloyd's reagent is washed once by shaking with about 15 ml. of 0.2 N sulfuric acid, centrifuged, and the wash acid decanted. Ten ml. of 0.5 N sodium hydroxide are now added, the contents made up to the mark with distilled water, and the tube again shaken for one minute and centrifuged. The colored supernatant fluid is decanted into another Pyrex tube (18 × 135 mm.) containing 0.6 g. of finely powdered lead nitrate, the tube shaken and centrifuged. The resulting, practically colorless, supernatant liquid is decanted into a third tube. In order to remove any excess lead, the solution is made slightly alkaline by the addition of small amounts of K_3PO_4 , using a single drop of phenolphthalein solution as indicator. The pH of the solution is then brought to approximately 4.5 by the cautious addition of 20 per cent orthophosphoric acid using Fisher Alkacid test paper or the glass electrode. The volume of fluid added must be kept to a few drops since the volume relationship is here being disturbed. The addition of two or three drops, however, results in only an insignificant error. The tube is centrifuged to throw down the new precipitate, and the supernatant fluid decanted for the König reaction.

Reagents for chemical reaction: Cyanogen bromide: A saturated solution of bromine water is titrated, in an ice bath, with 10 per cent sodium cyanide solution to complete disappearance of color, avoiding an excess of sodium cyanide. As an alternative method a 4 per cent aqueous solution of crystalline CNBr may be prepared. Either solution is stable for several weeks in the icebox.

Ten per cent KH_2PO_4 solution.

Metol (p-methylaminophenol sulfate): A saturated solution (about 5 per cent) is prepared immediately before using.

Development of color: A 5-ml. aliquot of the extract, equivalent to one ml. of skim milk, is transferred to an Evelyn macro-colorimeter tube. One ml. of 10 per cent KH_2PO_4 solution and 14 ml. of distilled water are added, making the volume 20 ml. This tube is designated as the *extract blank*. A second 5-ml. aliquot of the extract is placed in a large test tube, designated as *color development tube*, and 1 ml. of 10 per cent KH_2PO_4 and 3 ml. of water are added, making the volume 9 ml. To a second large test tube, designated as the *reagent blank*, is added 1 ml. of 10 per cent KH_2PO_4 and

8 ml. of water, making the volume 9 ml. These last two tubes are then placed in a water bath at 75–80° C. for five minutes. One ml. of cyanogen bromide solution is added to the warm tubes and they are again placed in the water bath at 75–80° C. for five minutes. The tubes are cooled under running water and 10 ml. of freshly prepared saturated metol solution are added to each, bringing the volume to 20 ml. They are then allowed to stand in the dark for one hour. At the end of this time the contents of the tubes are transferred to Evelyn macro-colorimeter tubes and all three tubes are read in the Evelyn photoelectric colorimeter. The readings are made using the No. 400 filter with the galvanometer set to read 100 with distilled water.

Calculations: The L values corresponding to the galvanometer readings for the three tubes are obtained from the chart accompanying the Evelyn Photoelectric Colorimeter. These L values are proportional to the optical density of the solutions. The L value obtained for the color development tube may be ascribed to three sources: the color of the extract, the color of the reagents, and the color resulting from the nicotinic acid reaction. An L value corresponding to the single factor, the quantity of nicotinic acid present, is obtained by subtracting the L value of the extract blank and the L value of the reagent blank from the L value of the color development tube. The number thus acquired, divided by the factor 0.0225, yields the nicotinic acid concentration in micrograms per ml. The factor, 0.0225, was obtained from standard solutions.

The procedure as outlined here is such that the final tube contains the equivalent of 1 ml. of skim milk. If a sample of a different size is taken, or if different dilutions are used, a dilution factor must also be introduced.

RESULTS AND DISCUSSION

Hydrolysis is carried out by digestion with hydrochloric acid. Dann and Handler (2) have pointed out the necessity for using rather small samples, about 0.5 g., for this digestion, in the analysis of tissue. The use of larger samples of certain tissues yields apparent values which are smaller as the size of the sample is increased. A similar behavior is found in analysis of milk. For example: with one sample from which 10 ml. and 5 ml. portions were taken for digestion, the estimated nicotinic acid was 1.16 and 1.34 $\mu\text{g.}$ per ml., respectively, with a second sample, 1.69 and 1.72, and with a third sample, 1.33 and 2.02. With samples smaller than 5 ml. the total amount of nicotinic acid involved in the color development is too small to obtain an estimate with any degree of accuracy.

As was pointed out by Perlzweig, Levy, and Sarett (13) and by Dann and Handler (2), it is essential, in the determination, to obtain almost complete decolorization of the extract. This point is illustrated in table 1 in which a comparison is given of the estimated nicotinic acid content of a sample of milk when almost complete and when only partial decolorization

was obtained with the Lloyd's reagent and lead salt treatment, and also when charcoal was used as the decolorizing agent. The differences in degree of decolorization, using the Lloyd's reagent and lead salt treatment, were obtained by varying the thoroughness with which the lead nitrate was mixed with the extract. The amount of colored material remaining in the extract is shown by the L value of the extract blank. When the color remaining in the extract blank gives an L value much greater than 0.0223, unreliable results are generally obtained.

TABLE 1

The effect of incomplete decolorization upon the estimation of nicotinic acid
(All determinations are on the same sample of milk)

Decolorizing agent	L value of extract blank	Estimated nicotinic acid <i>μg. per ml.</i>
Lloyd's and lead	0.0223	1.59
Lloyd's and lead	0.2366	4.44
Lloyd's and lead	0.4440	6.16
Charcoal (av. of 4 determinations)	0.1265	7.01

In this connection it is of interest to note the report of Melnick and Field (12) with regard to the advisability of addition of the aromatic amine to the extract blank. These authors point out that, although the addition of the amine to the extract blank results in the development of a color indistinguishable from that obtained with nicotinic acid, these side reactions do not occur in the presence of cyanogen bromide in the actual development of color for the nicotinic acid. They state that, consequently, the amine should not be added to the extract blank. Melnick and Field (12) report the results for milk, estimated by the two methods, as follows: with addition of the amine (aniline) to the extract blank, 0.12 mg. per cent; without addition of the amine, 0.44 mg. per cent. Expressed in micrograms per ml. the values are 1.2 and 4.4, respectively.

By the method used in the present study, the interfering substances involved are removed from the extract by the Lloyd's reagent and lead salt treatment, and the average value obtained is 1.46 $\mu\text{g. per ml.}$ This value agrees more closely with the value obtained by Melnick and Field (12) when the amine was *added* to the extract blank. It is somewhat lower than values obtained by Kodicek (9), using the method of Harris and Raymond (7), in which the amine (p-aminoacetophenone) is added to the extract blank. The values obtained by Kodicek are 1-5 $\mu\text{g. per ml.}$ with an average of 3.

Recovery of nicotinic acid added to milk is shown in table 2.

The data obtained from determinations on 24 samples of milk, run in quadruplicate, were analyzed statistically. This analysis gives the standard error of the means of four determinations to be 0.105, or 7.2 per cent of

TABLE 2
Recovery of nicotinic acid

Sample No.	No. of determinations	µg. per ml. added	µg. per ml. found	Per cent of total nicotinic acid found
I	4	0	0.94
	2	2	2.93	99.7
	2	4	4.64	93.9
II	6	0	1.67
	2	1	2.64	98.9
	2	2	3.88	105.7
	2	4	5.44	95.9

the average normal value obtained, 1.46. The limits of accuracy of the method seem satisfactory when one considers the small amounts of nicotinic acid present and the complexity of the determination.

The results of weekly analysis, during the month of January, 1941, of the milk of six Ayrshire cows from the North Carolina Experiment Station herd are given in table 3. Cows 1-5 were maintained on stock rations and the samples taken at the time of regular milking. Cow No. 6, which was in the eighth month of gestation, was not being milked regularly, and only sufficient milk for the nicotinic acid determinations was obtained. The values obtained from determinations on the milk of cow No. 6 are not included in the average value for normal cows. Each value in the table is an average of four determinations. The average for normal cows during this period was 1.46 micrograms per ml.

TABLE 3
Nicotinic acid content of milk from Ayrshire cows on stock ration

Date	Cow No. 1	Cow No. 2	Cow No. 3	Cow No. 4	Cow No. 5	Cow No. 6
	µg./ml.	µg./ml.	µg./ml.	µg./ml.	µg./ml.	µg./ml.
Jan. 7	2.12	1.42	1.48	1.32	1.40	2.16
Jan. 13	1.39	1.42	1.60	1.57	1.80	2.78
Jan. 20	1.38	1.12	1.47	1.47	1.89	2.26
Jan. 27	1.45	1.66	0.81	1.00	1.42	2.33
Average	1.58	1.40	1.34	1.34	1.63	2.38

Average of normal cows, Nos. 1-5, 1.46.

A comparison of the results obtained in this study with some of the values obtained by chemical methods, as reported in the literature, is given in table 4.

We suggest that the results of Melnick and Field (11) and of Kodicek (9) are too high due to incomplete decolorization of the extract.

The low values obtained are of interest in view of the fact that milk has long been considered of great importance in a pellagra-preventing diet.

TABLE 4
*Comparison of values obtained by different investigators for the
 nicotinic acid content of milk*

		µg. per ml. fresh milk
Swaminathan (14)	Skim milk powder (10.53 mg.%)	0.90-1.00*
Melnick and Field (11)	Fresh milk (.45 mg.%)	4.5
Kodicek (9)	Fresh milk (1-5 µg./ml., average 3)	3
Kodicek (9)	Dried milk (25 µg./g.)	2.88-3.62†
Present study	Fresh skim milk	1.46

* Skim milk powder 8.5-9.5% of original skim milk (16).

† Whole milk powder 11.5-14.5% of original milk (16).

Goldberger and his collaborators (4, 5, 6, 15) have found milk to give partial protection and sometimes complete protection from blacktongue in dogs and pellagra in man for periods as long as a year when preventive tests were made. In one pellagra test, complete protection was afforded by 40 ounces of buttermilk daily, yet on the basis of our figures for milk and those of Dann and Handler (2) for cornmeal, it is clear that milk contains less nicotinic acid than the equi-caloric quantity of cornmeal which must have been displaced from the diet.

There are two possible explanations of this paradox. First, that the chemical method of estimating nicotinic acid in milk fails to detect all the nicotinic acid. Second, that when considerable quantities of milk are included in the diet, the intestinal flora is so altered that a significant amount of nicotinic acid is synthesized by the intestinal microorganisms, and then absorbed from the intestine. The second suggestion seems to us to be much more probable than the first.

SUMMARY

A method is given for the estimation of nicotinic acid in milk.

The results of weekly analysis of the milk of six Ayrshire cows during the month of January, 1941, are given. The average nicotinic acid content of the milk from normal cows is found to be 1.46 micrograms per ml.

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THE LETHAL EFFECTIVENESS OF ULTRA VIOLET RAYS APPLIED TO MILK*

G. C. SUPPLEE, G. E. FLANIGAN AND O. G. JENSEN

*The Borden Company, Biological and Chemical Research Laboratories,
Bainbridge, New York*

Biologists have long recognized ultra violet radiation as a lethal agent for the destruction of bacteria. Its application for sterilization or the reduction of bacterial contamination of various substances is limited however, by the low penetrating power of the active radiation. The common gases of the air, water vapor, substances in solution, colloidal suspensions, and obviously solids, exert a general or specific screening effect on the active rays. As a consequence, such bacterial reduction as may be accomplished by spectral energy of this character must take place at or near the surface. Accordingly, one of the essentials in any problem of adaptation of this lethal principle is to provide surface exposure. The inherent principles operative in the mechanics of flowing fluid films of certain types, present a degree of surface exposure warranting studies designed to determine the utility and limitations of ultra violet energy for the destruction of bacteria in milk. The desirability of such experimentation has also been accentuated by the availability of improved and more potent emission sources. Pertinent results from practical but well controlled techniques obtained from commercial milk representative of the day to day receipts from a country receiving plant, are recorded hereinafter.

EXPERIMENTAL

One of the basic features of the technique employed in these studies involves flowing milk films, the characteristics of which could be duplicated at will by the simple expedient of originating the film from a horizontal capillary slot contiguous to the supporting surface and positioned to allow exit flow at an angle to the force of gravity. Flowing films thus projected onto a smooth surface can be controlled to a precise degree in regard to thickness during a given distance of travel, film capacity or rate of flow and uniformity of flow characteristics at the free interface. Speed of travel may also be controlled by varying the capacity and thickness of the film and the temperature. Figure 1 illustrates the equipment used for forming films with a 50-inch vertical travel and width up to 18 inches. This unit is provided with a baffled water jacket permitting regulation and control of the temperature of the flowing film if desired. Figure 2 illustrates similar equipment

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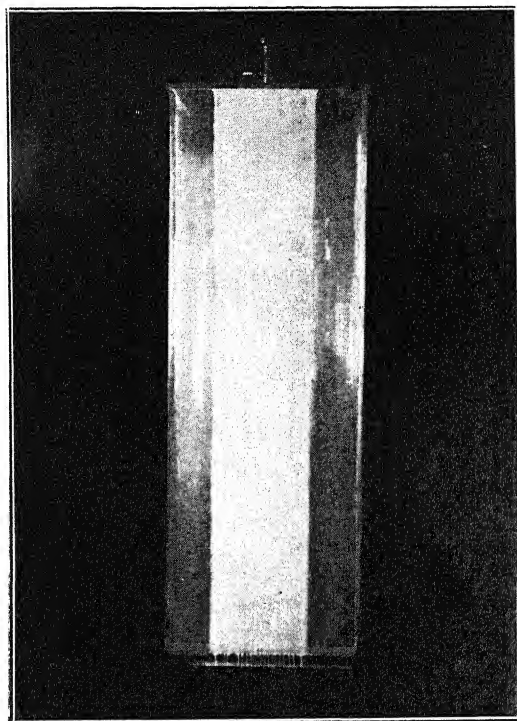


FIG. 1. Smooth flowing film used for determining lethal effect of ultra violet rays.

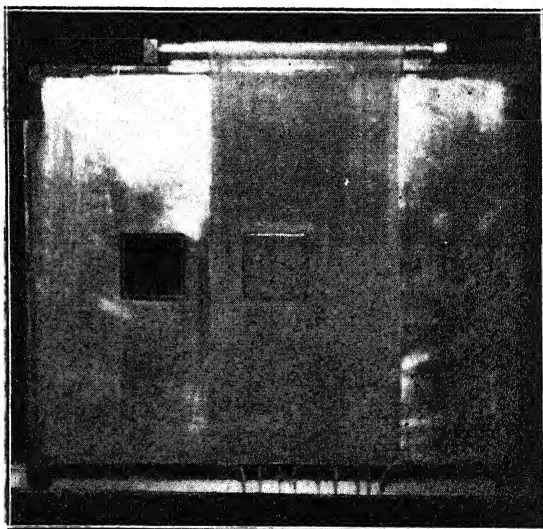


FIG. 2. Milk film flowing over quartz window used for determining comparative transmission of ultra violet rays.

provided with quartz windows for determining ultra violet or other radiation transmitted through the flowing film as it passes over the quartz window.

The sources of ultra violet radiation were mercury arcs and lamps of standard or special construction furnished by The Westinghouse Electric and Manufacturing Company. The arc tubes were approximately 22 inches long and one-half inch in diameter. These tubes, when used singly, were placed vertically in front of the flowing milk film at various distances depending upon the experimental plan, and positioned equi-distant laterally from the edges of the film and equi-distant longitudinally from the top and bottom. When a plurality of arcs were used, they were positioned horizontally with equal spacing between the tubes depending upon the number used and the length of the film irradiated.

Each arc operating at a given voltage and amperage emitted radiation of different spectral character, qualitatively and quantitatively. The relative intensity of the emissions from the different arcs, as measured through quartz and a two inch aperture by a metered tantalum photoelectric cell, (1, 2), was of the comparative magnitude of 148, 87, 18 and 4 at a 12-inch distance. The peak of sensitivity of this particular photo-cell was in the region, of 2550-2600 Å, with very slight registration as low as 2000 Å and a cut-off at approximately 3000 Å. By placing the photo-cell back of the quartz window over which milk was allowed to flow, as a smooth film (fig. 2), the intensity of the radiation transmitted by films of different capacity was readily obtained.

Chart 1 shows typical results obtained from each of the radiation sources studied, from which it is to be noted that there is substantially a straight line relationship correlating the intensity of the transmitted radiation with the film capacity, when employing logarithmic scales. It will also be noted that the order of magnitude of the transmitted radiation is not proportional to the emission intensity of the different arcs. This is accounted for by the fact that the spectral quality of each arc is of different character and the milk films selectively absorb the incident radiation in varying degrees. The salient characteristics of the radiation from the different sources may be briefly summarized as follows: Arc No. 1 with a radiation intensity of 148 has a strong 2537 Å emission, as high as 85 to 94 per cent of the total output; arc No. 2 with a radiation intensity of 18 has substantially the same characteristics as arc No. 1, but at less intensity, practically no short wave radiation is emitted by this arc however; arc No. 3 has substantially the same characteristics as arc No. 2 but with less intensity; arc No. 4 with a radiation intensity of 87 yields substantially none of the 2537 Å wave length but does emit very strong radiation in the 2200-2300 Å region, probably 80 per cent or more of the total energy output.

The bacteriological data summarized hereinafter were obtained over a period of years. All milks involved in the studies were representative of

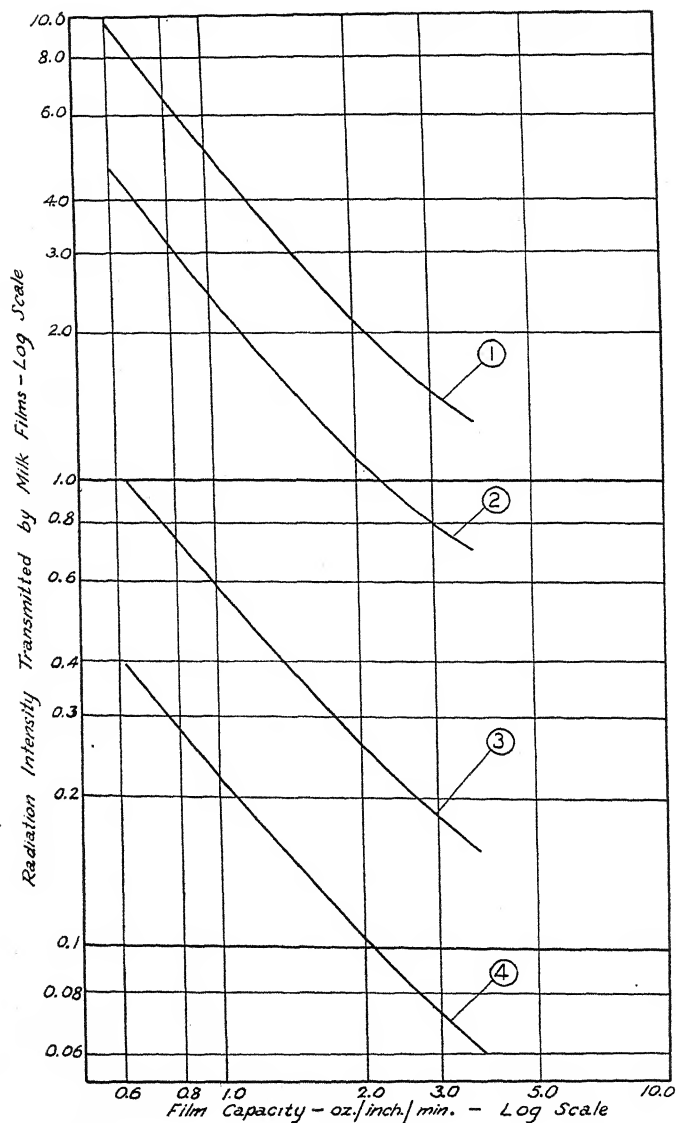


CHART 1. Transmission of ultra violet radiation through milk films. (Tantalum photo cell measurements.) 12 inches from arc to quartz or milk.

Are (1) radiation intensity	148
Are (2) " "	18
Are (3) " "	4
Are (4) " "	87

the daily receipts of mixed milk from a "Grade B" country plant in New York State. The composited records shown in graphical form are made up from hundreds of determinations with numerous different samples con-

tributing to a single result in the comparable relationships shown in the individual graphs. Since our objective was a thorough appraisal of the lethal value of ultra violet radiation applied to commercial milk, rather than a desire to demonstrate extreme results under conditions which would hold no promise of reduction to practise, it is believed that the graphical presentation most satisfactorily establishes the merits of the principles under investigation.

For all test runs at least two or three control samples of the untreated milk were taken from the supply tank located immediately above the film-forming member of the apparatus during the period of operation. Subsamples of the treated milk, frequently five or more, were taken at intervals during the course of the individual run. All platings were made in triplicate with Tryptone Glucose Agar, incubated at 37° C. In order to avoid discrepancies due to latent effects following irradiation, platings were made immediately and in practically all instances the agar was in the plates within three to five minutes after treatment.

The comparative degree to which differences in the spectral character and intensity of the various radiation sources were lethally effective when applied to milk films under conditions previously described, is shown in chart 2. The relative bactericidal results shown by graphs 1, 2, and 3 fall in the expected order as might be anticipated from the intensity of the incident radiation. Graph No. 4 obtained with the arc having a comparatively strong radiation intensity of 87 as measured by the tantalum photo-cell, shows a relatively inefficient bactericidal effect at the lower film capacities, but an unexpectedly high efficiency at the higher film capacities. Since this arc emits a high proportion of relatively short irradiation in the 2200–2300 Å region, other detailed studies with this source were carried out, typical results from which are shown in chart 3.

Graph No. 1 of chart 3 was constructed from data obtained by exposing a flowing film maintained at uniform capacity during a sequence of irradiation periods until a total exposure time of 28 seconds had accumulated. The initial exposure period of 8 seconds resulted in a greater reduction in bacteria count than was shown after more extended periods. This phenomenon was entirely contrary to any experience encountered with the other radiation sources. In order to study this matter further, stationary films of the same milk were prepared by allowing the film-supporting surface to drain until only a thin adhesion layer substantially 0.02 mm. in thickness, remained. Graph No. 2 in chart 3 shows the results obtained upon exposing such films to the short wave radiation for periods up to 34 seconds. The results generally confirm those obtained by irradiation of the flowing films, a greater lethal effect being manifested during the first few seconds exposure, this reduction in count being nullified on longer exposure with counts even in excess of the original untreated milk, after a period of 12 to 20 seconds.

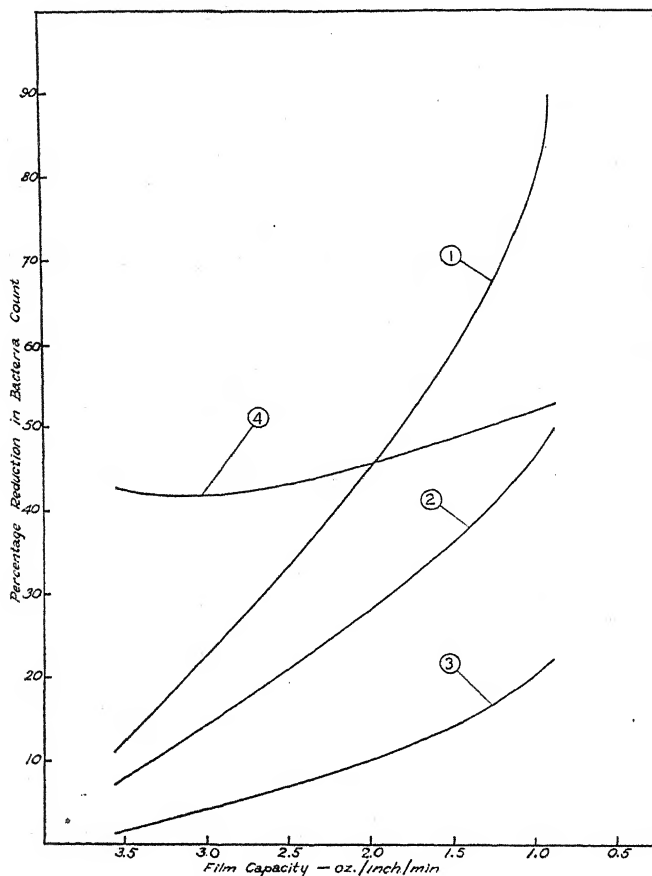


CHART 2. Lethal effect of different radiation sources applied to milk films.

Distance of film flow	50 inches
Film width	16 inches
Are to film	12 inches
Exposure time (varies with film capacity)	3.0-10 secs.
Film thickness (varies with film capacity)	
Curve (1) Arc radiation intensity	148
Curve (2) " " "	18
Curve (3) " " "	4
Curve (4) " " "	87

Thereafter an increase in lethal effectiveness as manifested by reduction in count became apparent. The full explanation of these observations with adequate supporting data is not now available. It is significant, however, that they support in principle, the observations of Coblenz and Fulton (3) and Hollaender *et al.*, (4, 5, 6) who have reported evidence of a bacteria stimulating effect resulting from sub-lethal doses of monochromatic ultra violet radiation. The relationships shown in the graphs could not be dupli-

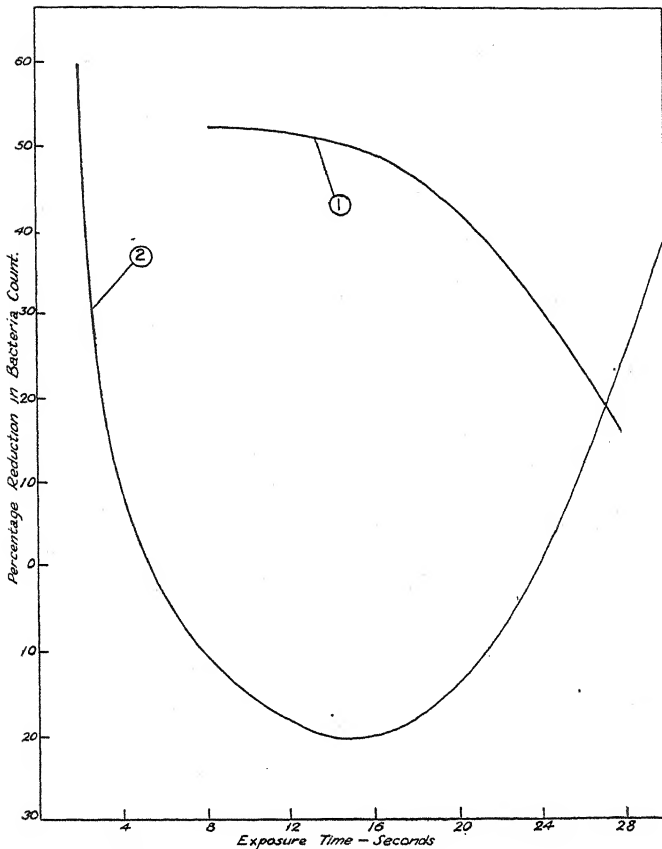


CHART 3. Lethal effect of short ultra violet radiation (arc No. 4) on stationary and flowing milk films.

	(1) Flowing films	(2) Stationary films
Film surface	Smooth	Smooth
Distance of film flow (ins.)	50-150	None
Film width (ins.)	3	3
Film capacity (oz./ins./min.)	1.62	None
Radiation source	Arc No. 4	Arc No. 4
Arc to film (ins.)	3.25	3.25
Film thickness (mm.)	0.2-0.25	0.02

cated at will from time to time with different milks. In some instances an increased count in the flowing film represented a two-fold increase in the count of the original milk; in a few instances a progressive decrease in count was obtained with increasing exposure time; and in other cases reduction in counts up to 90 or 95 per cent was obtained on momentary exposure of only a few seconds with no evidence of subsequent increase in count upon continued exposure.

These characteristically erratic results were generally obtained with the arc emitting a high proportion of short wave lengths in the absence of 2537 Å radiation. Such wave lengths are absorbed at the free interface of the flowing film to a much greater degree than longer wave lengths. It may be postulated therefore that since the lethal effect of short radiation is known to be greater than that of longer wave lengths provided it reaches the organism, the destructive action noted took place at or substantially at the free interface. Whereas, those organisms below the free interface received only sub-lethal doses, but did receive an increment of screened radiation sufficient to stimulate greater viability and growth in artificial media. This evidence of apparent stimulation may have been due in fact, to the jarring apart of aggregates of bacteria without destruction of the individual organism. Such a conjecture, however, is not supported by the observations by other investigators to which reference has already been made.

It is not to be assumed that the erratic results obtained from the short ultra violet radiation are characteristic examples of the bactericidal efficacy of all ultra violet energy when appropriately applied to milk. A relatively high and consistent reduction in bacteria count may be obtained under conditions readily duplicable at will. The degree of bacterial reduction in milk is, however, dependent not only upon the character and intensity of the incident radiation, but also upon the manner in which it is applied. Certain principles which influence its lethal effectiveness are exemplified by the data which follow.

Chart 4 illustrates the relative bactericidal effect obtained by a plurality of arcs (No. 3) with the weakest radiation intensity of any of those used throughout this work. Bacterial reduction is correlated with the time of exposure of flowing and stationary films, the latter results being included as a basis of reference and comparison. The flowing films (graph No. 2) show an average percentage reduction varying from 35 per cent to in excess of 95 per cent during exposure periods from about 2.5 seconds to 16 seconds, the capacity of the films being inversely correlated with the exposure time. These results, as compared with those obtained from the stationary films exposed for comparable periods and which represent an adhesion layer only (graph No. 1), are of significance in illustrating inherent principles wherein the effect of the reactive energy is confined to, or near the surface. Notwithstanding the substantially thinner layer of milk exposed as a stationary adhesion film, the reduction in bacterial content is commensurately less than was obtained in the flowing film of much greater thickness, for comparable time exposure of the milk mass. It is to be concluded therefore that the flowing films inherently present a greater exposure per unit volume at the free interface than the stationary films, thus the lethal property of the incident radiation is more effectively utilized. Objective results of a practical character in which this principle is operative have also been shown in the

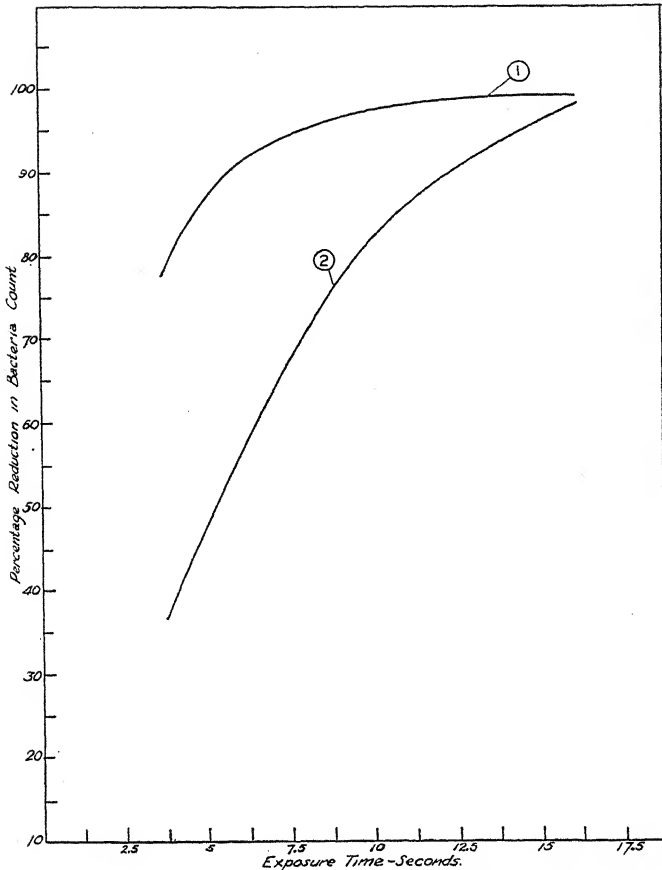


CHART 4. Lethal effect of ultra violet radiation on stationary and flowing milk films.

	(1) Stationary films	(2) Flowing films
Film surface	Smooth	Smooth
Distance of film flow (ins.)	None	50
Film width (ins.)	16	16
Film capacity (oz./in./min.)	None	0.9-3.6*
Radiation source	Are No. 3 (20 units)	Are No. 3 (20 units)
Are to film (ins.)	3.25	3.25
Film thickness (mm.)	0.02	0.2-0.4†

* Varies with exposure time.

† Varies with film capacity.

irradiation of milk for vitamin D activation (7). The results shown by these data illustrate primarily, a principle influencing the lethal effectiveness of ultra violet radiation as applied to milk, but do not represent the greatest degree in bacterial reduction which may be obtained by this means. However, before presenting other considerations, data obtained from films flowing over smooth and corrugated surfaces should be examined.

Chart 5 shows the comparative bacterial reduction in milk films of varying capacity when exposed as a smooth film projected as heretofore described and as a film flowing over a standard two-inch tubular cooler surface. The distance of vertical travel of the smooth film was 27.5 inches; the projected

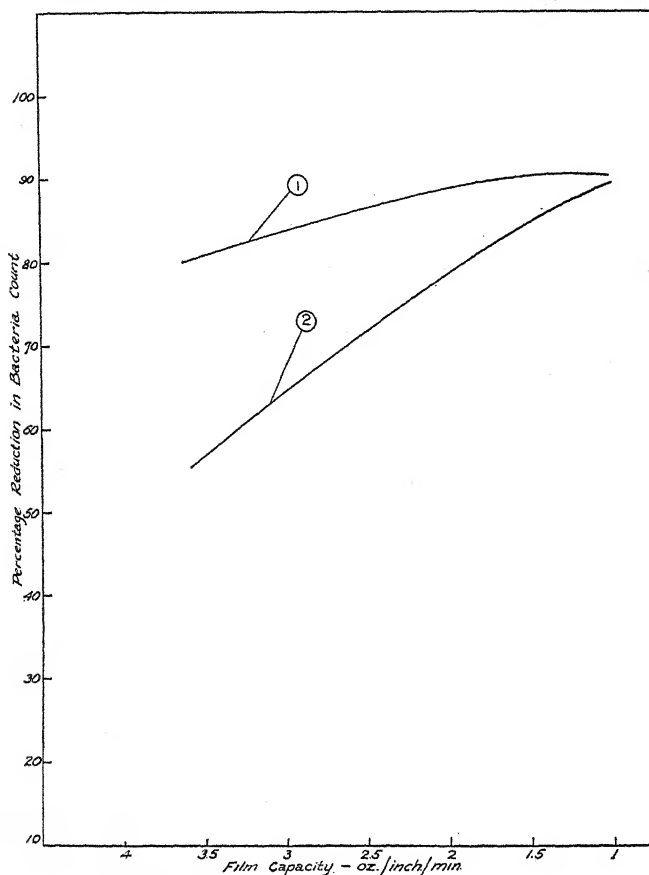


CHART 5. Lethal effect of ultra violet radiations on smooth and corrugated films.

	(1) Smooth films	(2) Corrugated films
Film surface	Smooth	Corrugated*
Distance of film flow (ins.)	27.5	41.5†
Film width (ins.)	16	16
Film capacity (oz./in./min.)	Variable	Variable
Radiation source	Arc No. 3 (12 units)	Arc No. 3 (12 units)
Arc to film (ins.)	3.25	3.25
Exposure time (secs.)	Variable	Variable
Film thickness (mm.)	Variable	Variable

* Cooler surface.

† 27.5 inches projected vertical distance.

vertical length of the corrugated surface was also 27.5 inches. However, the actual distance of film travel over the corrugated surface was 41.5 inches, or substantially 50 per cent greater than the distance travelled by the smooth film. It might be expected that this greater distance of film travel would contribute to greater exposure per unit of volume at the free interface and thus enhance the lethal effectiveness of the incident rays, causing a greater reduction in bacteria count. However, an examination of the graphs in chart 5 shows the reverse to be true, a greater reduction in count being obtained from the smooth films traveling a lesser distance than the corrugated films.

One of the reasons for this result is probably illustrated by the scale diagrams shown in chart 6. Figure 1 shows the variable and irregular thick-

FIGURE-1

FIGURE-2

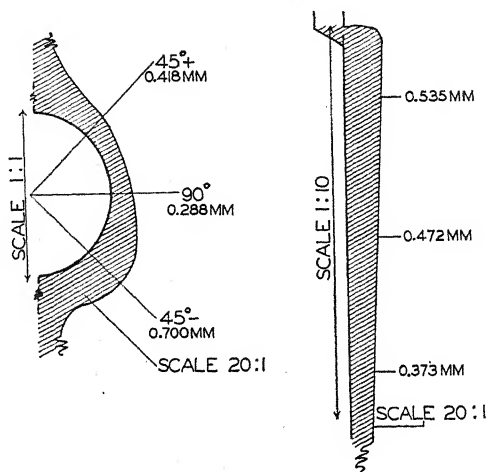


CHART 6. Thickness of flowing milk films as affected by contour of surface. (Temperature 68-70° F.; Film capacity 3.55 oz./inch/min. ± 0.15 .) Figure 1. Two-inch tube of cooler assembly. Figure 2. Smooth metal surface—50 inches. (Film from capillary slot at right angle to gravity.)

ness of the film traveling over a two-inch corrugation of a tubular cooler at a distance approximately 10 inches from the point of origin. It is to be noted that the transverse section of the film tends to assume a characteristic rain-drop shape with the thinner section of the film at, or a little above, the horizontal plane of intersection, whereas the thicker portion is about 45° below the horizontal intersection. Comparison of the shape of the transverse section of the film flowing over the two inch contour, with the uniform and progressively tapering wedge-shaped transverse section of the film projected from the capillary slot, flowing over a smooth surface, (figure 2) readily shows that greater surface exposure at the interface per unit volume does

not necessarily prevail in the corrugated film, notwithstanding the greater linear distance traveled. An analysis of the interacting forces of gravity, friction and surface tension readily reveal the reasons for the differences in thickness and character of the two types of films. [The actual film thickness measurements were made by the method described by Beck (8).] A further explanation of the lower bactericidal result obtained with the corrugated films is probably due to the fact that a higher proportion of the energy impinged on the surface at angles substantially less than normal, a condition which increases reflection and otherwise reduces the efficiency of the incident radiation.

Since ultra violet radiation is a form of energy, as is heat, it is desirable to compare the bactericidal effectiveness of each of these forms of energy as applied to milk; such comparative data are shown in charts 7 and 8. Graph No. 1 of chart 7 shows a normal percentage reduction curve obtained by treating milk in the Electro-pure pasteurizer at various temperatures from 162° F. to 195° F. The percentage reduction figures through this range increase progressively from about 96 per cent to 99.9 per cent. These same heat treated milks, cooled, and subsequently subjected to ultra violet radiation from arc No. 1 with a radiation intensity of 148, show a slight but measurably consistent further reduction in bacteria count (computed to the original raw milk basis) as shown in graph No. 2. However, the comparative efficiency of the ultra violet energy applied to the previously heated milks is more clearly revealed in graph No. 3. A substantially consistent 60 per cent reduction was effected by the radiant energy applied to the milks heated through the 162° F. to 185° F. temperature range. Thereafter the efficiency of the radiant energy fell off rapidly. These relationships and comparisons obviously present stimulating problems of a strictly bacteriological character, as it is apparent that certain species or individual organisms inherent in the normal milk flora are not completely eliminated by this integrated application of thermal and radiant energy.

Chart 8 shows the data obtained by irradiating milk with the most lethal energy source used throughout this work (arc No. 1), during elevation of the temperature to various levels throughout the range 80° F. to 180° F. Heating of the flowing milk film was accomplished by electrical means described elsewhere by Supplee and Jensen (9). The time of exposure to the radiant energy concurrent with the elevation in temperature for each of the milks did not exceed 0.7 seconds. Graph No. 1 shows the percentage reduction curve obtained by the thermal treatment only and represents, in so far as we are aware, the first correlated data of this character wherein the time factor is substantially eliminated. The influence of temperatures up to about 120° F. applied for a fraction of a second apparently caused an increase in bacterial count, but a lethal action prevails thereafter with progressing effectiveness as the temperature is increased. Graphs No. 2 to No. 5

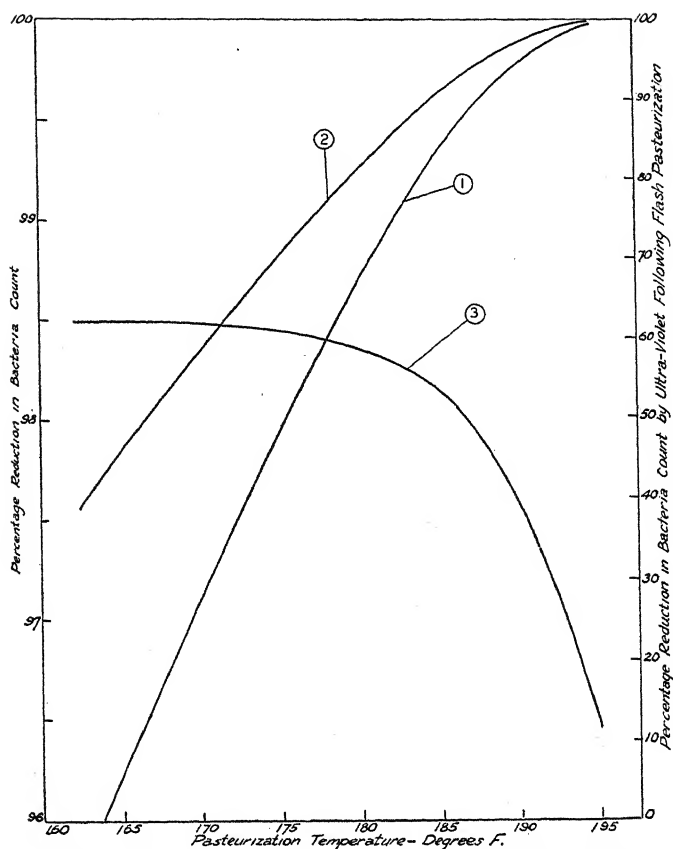


CHART 7. Lethal effect of ultra violet radiation following flash pasteurization.

- (1) Flash pasteurization (Electro pure apparatus).
 (2) Flash pasteurization (Electro pure apparatus) followed by ultra violet radiation.

Film surface	Smooth
Distance of film flow (ins.)	50
Film width (ins.)	16
Film capacity (oz./in./min.)	1.62
Radiation source	Arc No. 1
Arc to film (ins.)	12
Film thickness (mm.)	0.2-0.25
Exposure time (secs.)	7.6

- (3) Lethal effect of ultra violet on flash pasteurized milk.

represent the results obtained from each of the four different radiation sources wherein their respective emissions were applied during elevation of the temperature. These results indicate that the combined lethal effect of the two forms of energy is primarily additive, the increment of the total bactericidal result contributed by each becoming apparent only as the other component contributes a secondary or lesser effect. This relationship is best

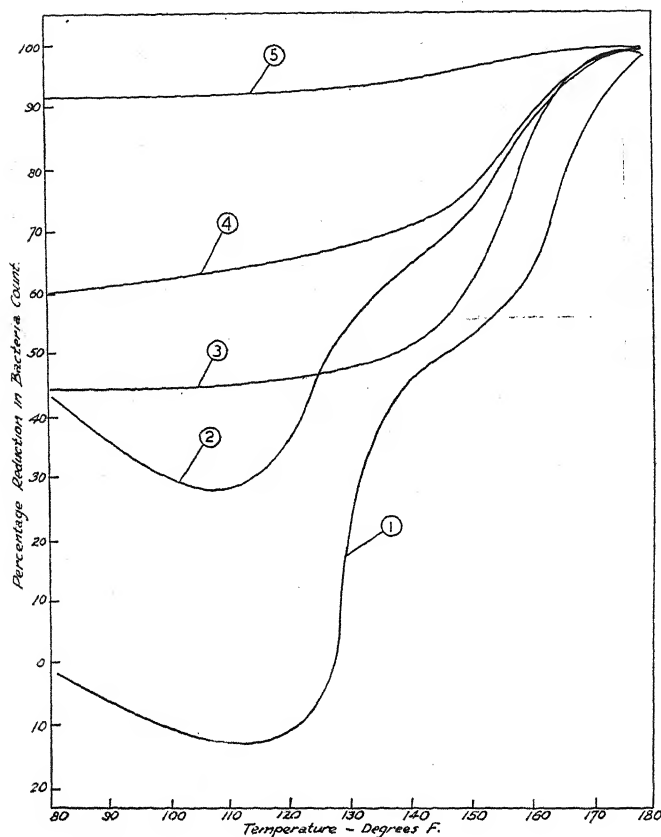


CHART 8. Lethal effect of ultra violet radiation simultaneously applied with flowing film heat treatment.

Distance of film flow (ins.)	12
Film width (ins.)	6
Film capacity (oz./in./min.)	3.6
Arc to film (ins.)	3.25
Exposure time (secs.)	0.7
Film thickness (mm.)	0.35-0.45

Curve (1) S-J Electric Heater

Curve (2) " " " "	+ Arc No. 3 (Intensity 4)
Curve (3) " " " "	+ Arc No. 4 (Intensity 87)
Curve (4) " " " "	+ Arc No. 2 (Intensity 18)
Curve (5) " " " "	+ Arc No. 1 (Intensity 148)

illustrated by a comparative analysis of graphs 1, 2 and 5. The substantially horizontal line of graph 5 representing 90 per cent reduction or more by the radiation from arc No. 1, especially throughout the range of lower temperature treatments in which range temperature alone caused an increase in counts, is typical of the degree of reduction, which has been consistently

obtained from this source throughout extended experiments in which no heat treatment was involved.

The various data and inter-relationships as presented illustrate certain principles involved in the study and application of ultra violet energy as a lethal agent for the destruction of bacteria in milk. Further refinement in the development of preferred methods of application must necessarily take these principles into account. The differences in the spectral characteristics of the radiations from the arcs included in this study, correlated with their lethal effectiveness as applied under the conditions described, clearly indicate a preferred specificity of energy distribution and intensity as essential for a high bactericidal effect when applied to milk.

SUMMARY

1. The lethal effectiveness of ultra violet energy applied to smooth flowing milk films is dependent upon the intensity and spectral characteristics of the radiation employed.

2. A greater bactericidal effect was obtained by ultra violet radiation applied to smooth flowing milk films than was obtained from films flowing over a corrugated cooler surface.

3. The effectiveness of the lethal radiation was relatively greater per unit volume of milk when applied to flowing films than when applied to stationary films having the thickness of an adhesion layer only.

4. Short ultra violet radiation predominantly in the 2200–2300 Å region gave erratic bactericidal results with evidence that sub-lethal doses may cause stimulation.

5. The total lethal effect of ultra violet energy and elevated temperatures simultaneously applied for less than one second appears to be an additive process.

6. Percentage reduction in bacteria counts of raw mixed milk in excess of 90 per cent and frequently up to 95 to 98 per cent may be obtained with a high degree of regularity by preferred methods of applying ultra violet radiation of appropriate intensity and spectral characteristics.

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REPORT OF THE STUDENTS' NATIONAL CONTEST IN JUDGING DAIRY CATTLE

Memphis, Tenn., October 11, 1941

The students' national contest in judging dairy cattle was held at the National Dairy Show, Memphis, Tennessee, on October 11. Twenty-four teams were entered.

The results of the contest were announced at a banquet held at the Gayoso Hotel on the evening of October 12. Dr. Jacob, Dean of the College of Agriculture of the University of Tennessee, and Chief O. E. Reed of the U. S. D. A. Bureau of Dairy Industry were the speakers. The Queen of the Dairy Show and her maids of honor attended the banquet.

The team from Iowa State College won the contest and were awarded permanent possession of the National Dairy Association cup as this was the third time they had won it. They were awarded the Hoard's Dairyman sweepstakes cup for the second time.

The Texas A. & M. College team placed second and were given possession for one year of the glory trophy awarded by the J. B. Ford Company.

Lyle Jackson was high man in the contest and received a gold medal from the National Dairy Association, a gold watch from the J. B. Ford Company, a pen and pencil set from the Country Gentleman and a cane from Successful Farming.

Lon McGilliard of Oklahoma placed second and received a radio from Starline, Inc., a silver medal from the National Dairy Association and a cane from Successful Farming.

Ralph Porterfield of Ohio placed third and received a bronze medal and a cane.

The Kansas team placed first in judging Ayrshires and were awarded the traveling trophy by the Ayrshire Breeders' Association, together with silver goblets. Russell Lyons of Iowa was high man. Ohio was first in Brown Swiss with Ralph Porterfield of Ohio high man. The winning team received a new traveling trophy with a built-in Swiss bell. The members of the team and high man received pen and pencil sets. Iowa placed first in judging Guernseys with J. B. Miller of Mississippi high man. The team was awarded the large traveling silver Guernsey cream can and the members of the team and high man received small cream cans. The Georgia team placed first to win the Holstein-Friesian Association of America silver cup and with the high man, Jack Paulson of Nebraska, were awarded gold medals. The team from Kansas was first in judging Jerseys, winning the bronze Jersey cow together with gold medals for the team. To Oscar Wesson of Purdue, the high man in Jerseys, went the coveted \$300.00 scholarship from the American Jersey Cattle Club.

The following is a list of the individuals and teams who won high standings in the contest:

ALL BREEDS

INDIVIDUALS

1.	Lyle Jackson, Iowa State College	1804.4
2.	Lon McGilliard, Oklahoma A. & M. College	1795.9
3.	Ralph Porterfield, Ohio State University	1791.8
4.	H. B. Hale, Texas A. & M. College	1768.8
5.	J. F. Cavanaugh, Kansas State College	1762.8
6.	Jack Paulson, University of Nebraska	1760.8
7.	J. D. Miller, Mississippi State College	1760.4
8.	Max Dawdy, Kansas State College	1757.2
9.	Oscar Wesson, Purdue University	1734.6
10.	Alfred Huseeth, University of Minnesota	1714.0

TEAMS

1.	Iowa State College	5183.4
2.	Texas A. & M. College	5168.9
3.	Kansas State College	5141.7
4.	University of Nebraska	5114.4
5.	Oklahoma A. & M. College	5045.8
6.	Georgia State College	5036.4
7.	University of Kentucky	4981.8
8.	Ohio State University	4972.0
9.	Mississippi State College	4925.2
10.	University of Minnesota	4895.9

AYRSHIRES

INDIVIDUALS

1.	Russell Lyons, Iowa State College	381.7
2.	William Hastnell, University of Nebraska	381.3
3.	J. F. Cavanaugh, Kansas State College	380.6
4.	H. B. Hales, Texas A. & M. College	379.6
5.	Walter Harvey, Oklahoma A. & M. College	379.1
6.	J. C. Kay, Texas A. & M. College	378.9
7.	Jack Paulson, University of Nebraska	378.6
8.	J. R. Weis, Kansas State College	378.4
9.	Alfred Huseeth, University of Minnesota	378.0
10.	Lyle Jackson, Iowa State College	376.7

TEAMS

1.	Kansas State College	1133.9
2.	University of Nebraska	1125.4
3.	Iowa State College	1116.8
4.	Oklahoma A. & M. College	1110.2
5.	Texas A. & M. College	1104.8
6.	University of Minnesota	1097.7
7.	Purdue University	1097.0
8.	New Jersey State College	1096.5
9.	University of New Hampshire	1093.1
10.	Texas Technological College	1088.9

BROWN SWISS

INDIVIDUALS

1.	Ralph Porterfield, Ohio State University	382.2
2.	Harold Hansen, University of Nebraska	360.3
3.	Howard H. Smith, University of Illinois	354.1
4.	Lyle Jackson, Iowa State College	353.3
5.	Albert Hall, Michigan State College	353.0
6.	H. B. Hales, Texas A. & M. College	352.3
7.	Stokes Homan, New Jersey State College	351.2
8.	Lon McGilliard, Oklahoma A. & M. College	347.3
9.	Robert Russell, University of New Hampshire	346.7
10.	J. F. Cavanaugh, Kansas State College	346.5

TEAMS

1.	Ohio State University	1020.6
2.	Iowa State College	1012.9
3.	University of Nebraska	995.1
4.	University of New Hampshire	971.5
5.	Texas A. & M. College	967.5
6.	University of Kentucky	963.9
7.	Kansas State College	962.8
8.	University of Illinois	960.6
9.	Georgia State College	944.0
10.	New Jersey State College	939.6

GUERNSEYS

INDIVIDUALS

1.	J. D. Miller, Mississippi State College	385.0
2.	Lyle Jackson, Iowa State College	383.8
3.	Lon McGilliard, Oklahoma A. & M. College	382.6
4.	Gene Campbell, University of Missouri	379.8
5.	Melvin Hanson, University of Minnesota	376.7
6.	Russell Lyons, Iowa State College	369.9
7.	H. B. Hale, Texas A. & M. College	360.2
8.	Curtis Avery, Georgia State College	359.5
9.	D. L. Ator, Texas A. & M. College	355.8
10.	C. E. Friday, Mississippi State College	353.9

TEAMS

1.	Iowa State College	1093.9
2.	Texas A. & M. College	1057.9
3.	Mississippi State College	1048.0
4.	Oklahoma A. & M. College	1029.1
5.	University of Missouri	1028.6
6.	University of Minnesota	1004.6
7.	Kansas State College	992.6
8.	Georgia State College	992.3
9.	Pennsylvania State College	987.1
10.	University of Illinois	985.7

HOLSTEINS

INDIVIDUALS

1. Jack Paulson, University of Nebraska	378.7
2. D. L. Ator, Texas A. & M. College	374.6
3. Thomas Duffy, University of Kentucky	366.3
4. Austin Rheney, Georgia State College	363.7
5. Harry Barnes, Georgia State College	362.1
6. Ralph Porterfield, Ohio State University	358.5
7. Max L. Dawdy, Kansas State College	356.4
8. J. F. Cavanaugh, Kansas State College	353.4
9. R. B. Dawson, Jr., Texas Technological College	353.2
10. Bill Hartnell, University of Nebraska	352.2

TEAMS

1. Georgia State College	1071.0
2. University of Nebraska	1051.1
3. Texas A. & M. College	1030.7
4. University of Kentucky	1013.4
5. Kansas State College	999.5
6. Michigan State College (Tie)	978.1
7. Ohio State University (Tie)	978.1
8. Oklahoma A. & M. College	975.1
9. University of Tennessee	963.4
10. Iowa State College	958.0

JERSEYS

INDIVIDUALS

1. Oscar Wesson, Purdue University	383.0
2. Ralph Porterfield, Ohio State University	372.1
3. Max L. Dawdy, Kansas State College	369.6
4. Frank Smith, University of Missouri	360.4
5. Lon McGilliard, Oklahoma A. & M. College	360.1
6. Philip Lautenbach, University of Wisconsin	353.1
7. Edwin Stauffacher, University of Wisconsin	352.7
8. Harold Hansen, University of Nebraska	352.7
9. C. H. Smith, New Jersey State College	352.1
10. H. B. Hales, Texas A. & M. College	351.8

TEAMS

1. Kansas State College	1052.9
2. Ohio State University	1021.3
3. University of Nebraska	1013.8
4. Texas A. & M. College	1008.0
5. Iowa State College	1001.8
6. University of Tennessee	999.0
7. Oklahoma A. & M. College	994.1
8. Mississippi State College	965.2
9. Georgia State College	956.5
10. University of Maryland	954.7

REPORT OF THE STUDENTS' NATIONAL CONTEST IN JUDGING DAIRY PRODUCTS

Toronto, Canada, October 20, 1941

The Ohio State University team was again victorious in the Dairy Products Judging Contest held at the Dairy Industries Exposition in Toronto in which 22 teams participated. Second and third places were won by teams from the University of Connecticut and Michigan State College, respectively. These three teams were awarded the Dairy Industrial Fellowships furnished by the Dairy Industries Supply Association.

First ranking teams and first ranking individuals also in the judging of butter, cheese, ice cream and milk were from Connecticut, Michigan, Connecticut and Ohio, respectively. The highest ranking individual in the entire contest was from Ohio State University.

Gold, silver and bronze medals were awarded to the individual contestants who won first, second and third places respectively in the judging of each of the four products as well as for all products.

The fellowships, cups and medals were provided by the Dairy Industries Supply Association which has sponsored the contest since 1930. The contest held this year was the 25th contest in the judging of dairy products sponsored wholly or in part by the American Dairy Science Association.

The 22 teams in the 1941 contest represented University of Connecticut, Cornell University, University of Illinois, Iowa State College, Kansas State College, University of Maryland, Massachusetts State College, Michigan State College, University of Minnesota, Mississippi State College, University of Nebraska, Ohio State University, Ontario Agricultural College, Pennsylvania State College, Purdue University, South Dakota State College, University of Tennessee, Texas A. & M. College, Texas Technological College, University of Vermont, Virginia Polytechnic Institute and University of Wisconsin.

Following is a list of those who won high standings in the contest:

ALL PRODUCTS

INDIVIDUALS

1. John R. Kohl, Ohio State University	92.80
2. Arnold C. Smith, University of Vermont	93.15
3. Kenneth M. Dunn, Michigan State College	94.85
4. Fred W. Carver, Ohio State University	96.90
5. Saul M. Glick, Massachusetts State College	100.35
6. Kurt Huber, South Dakota State College	100.80
7. Leonard Goldberg, University of Connecticut	101.25
8. Douglas Dana, University of Vermont	102.05
9. Willim Rabinovitz, Massachusetts State College	102.50
10. Richard Marland, University of Connecticut	103.45

TEAMS

1. Ohio State University	13.00
2. University of Connecticut	21.00
3. Michigan State College	22.00
4. Massachusetts State College	29.00
5. University of Illinois	30.00
6. University of Vermont	35.00
7. Mississippi State College	37.00
8. Cornell University	38.00
8. Texas Technological College	38.00
10. Iowa State College	39.00

BUTTER

INDIVIDUALS

1. Leonard Goldberg, University of Connecticut	8.50
2. Burns E. Woodward, University of Nebraska	9.50
3. Mernice R. Volkens, Iowa State College	10.00
3. Harry Nohren, University of Illinois	10.00
5. Robert W. Bereiter, University of Wisconsin	10.50
5. Arnold C. Smith, University of Vermont	10.50
7. Howard O. Beach, Iowa State College	11.00
7. Richard Marland, University of Connecticut	11.00
9. Robert Crombie, University of Illinois	11.50
10. Fred W. Carver, Ohio State University	12.00
10. Dale W. Byers, Pennsylvania State College	12.00
10. Norman R. Stoddard, University of Vermont	12.00
10. Robert Edward Carr, University of Illinois	12.00
10. Millard A. Gillham, Texas Technological College	12.00

TEAMS

1. University of Connecticut	32.00
2. University of Illinois	33.50
3. University of Nebraska	37.00
4. University of Minnesota	41.00
5. Cornell University	41.50
6. Pennsylvania State College	42.60
7. Ohio State University	43.00
7. Iowa State College	43.00
9. South Dakota State College	44.00
10. Texas Technological College	45.50
10. University of Vermont	45.50

CHEESE

INDIVIDUALS

1. Robert E. Stout, Michigan State College	25.50
2. Clyde H. Stuntz, Iowa State College	27.00
3. Fred W. Carver, Ohio State University	28.75
4. Howard O. Beach, Iowa State College	29.75
5. Kenneth M. Dunn, Michigan State College	30.00
5. Arnold C. Smith, University of Vermont	30.00

5.	Richard Erickson, University of Minnesota	30.00
8.	Royal N. Gober, Mississippi State College	30.25
9.	Talwin Ruttum, South Dakota State College	30.75
10.	Glenn H. Whetzel, Purdue University	31.00
10.	Morris Van Daele, Kansas State College	31.00

TEAMS

1.	Michigan State College	87.50
2.	Ohio State University	93.00
3.	Mississippi State College	97.25
4.	University of Connecticut	98.50
4.	Iowa State College	98.50
6.	University of Wisconsin	100.50
7.	Massachusetts State College	101.00
8.	Cornell University	102.25
9.	South Dakota State College	103.50
10.	University of Illinois	103.75
10.	University of Minnesota	103.75

ICE CREAM

INDIVIDUALS

1.	Leonard Goldberg, University of Connecticut	29.50
2.	Kenneth M. Dunn, Michigan State College	29.75
3.	Henry Swain, Cornell University	30.25
4.	John R. Kohl, Ohio State University	30.75
5.	William Rabinovitz, Massachusetts State College	32.00
5.	Kurt Huber, South Dakota State College	32.00
5.	Saul M. Glick, Massachusetts State College	32.00
8.	Herman L. Neiditz, University of Connecticut	33.75
9.	Fred W. Carver, Ohio State University	35.25
10.	L. T. Simpson, Mississippi State College	35.75
10.	Arnold C. Smith, University of Vermont	35.75

TEAMS

1.	University of Connecticut	101.50
2.	Massachusetts State College	104.25
3.	Ohio State University	108.50
4.	Cornell University	113.00
5.	Michigan State College	116.75
6.	University of Maryland	119.00
7.	Purdue University	122.00
8.	University of Vermont	122.50
9.	Mississippi State College	128.50
10.	University of Illinois	132.00

MILK

INDIVIDUALS

1.	James R. Ebright, Ohio State University	15.00
2.	Clarence E. Kunz, Pennsylvania State College	16.15
3.	John R. Kohl, Ohio State University	16.55
4.	Arnold C. Smith, University of Vermont	16.90

5.	William Rabinovitz, Massachusetts State College	17.00
6.	Marion Smith, University of Tennessee	17.40
7.	Percy J. Smeltzer, Michigan State College	17.60
8.	Roy Lawson, University of Tennessee	18.10
8.	Roy Moffett, Texas Technological College	18.10
10.	Joe McGregor, Texas Technological College	18.55

TEAMS

1.	Ohio State University	52.45
2.	University of Vermont	59.50
3.	Texas Technological College	60.30
4.	Michigan State College	60.40
5.	Pennsylvania State College	61.10
6.	Massachusetts State College	62.05
7.	University of Tennessee	64.35
8.	University of Illinois	72.10
9.	Iowa State College	72.35
10.	Mississippi State College	74.80

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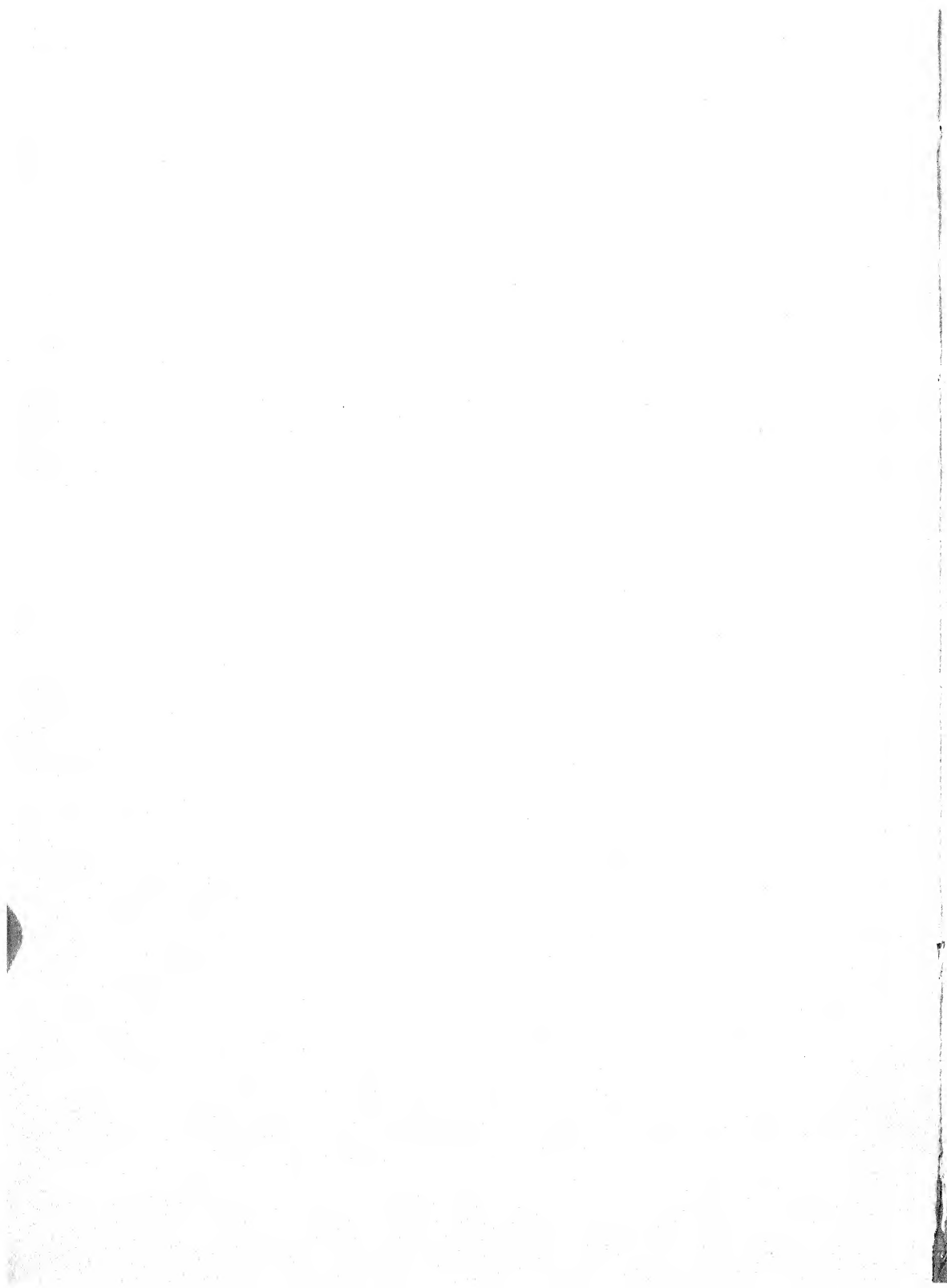
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ABSTRACTS OF LITERATURE

ADVANCE ABSTRACTS OF REPORTS ACCEPTED FOR PUBLICATION IN THE JOURNAL OF DAIRY SCIENCE

391. **Oxidation-Reduction Potentials and the Oxidized Flavor in Homogenized Milk.** P. B. LARSEN, I. A. GOULD AND G. M. TROUT, Michigan State College.

Oxidation-reduction potentials were determined both on unhomogenized and homogenized milk. In certain trials copper was added to the milk at the rate of 1 and 3 p.p.m. Homogenization was at 1500 and 2500 pounds pressure. The results showed that the Eh in both unhomogenized and homogenized milk exhibited the same trend whereas the milks showed differences in susceptibility to oxidized flavor development. Unhomogenized milk which became oxidized showed increases in potential. In contrast, the same milk when homogenized underwent similar changes in potential but showed little or no tendency to become oxidized.

392. **Effect of Certain Factors Upon Lipolysis in Homogenized Raw Milk and Cream.** I. A. GOULD, Michigan State College.

Fat from milk or cream homogenized at 500-1000 pounds pressure was titrated for free fatty acids. The influence of the following factors was observed: copper, salt (NaCl), formalin, storage temperature, and different fractions of milk. In addition, correlation between free fatty acids and formation of peroxides was determined. The results showed that neither copper salts in amounts as high as 10 p.p.m., nor formalin in ratio of 1:250, when added to the milk or cream, affected the speed nor extent of lipolysis. Salt markedly reduced lipolysis when added at the rate of two per cent, and almost completely prevented lipolytic activity when used at the rate of five per cent. Fat in homogenized raw milk underwent 12 fold more lipolysis at 70° F. than at 35° F., whereas the fat in the milk stored at 35° F. showed approximately twice as much hydrolysis as that in the milk held at 0° F. Pasteurization of cream and skimmilk fractions showed the active agent of lipolysis to be associated with the milk plasma. Further, precipitation of the casein by rennet and homogenizing fat in raw whey, showed that the whey exhibits definite lipolytic activity, although the major portion of the activity of skimmilk was lost with the removal of the casein. No significant relationship was observed between the extent of lipolysis in the homogenized products and the peroxide number.

393. **Rancidity Studies on Mixtures of Raw and Pasteurized Milk.** P. B. LARSEN, G. M. TROUT, AND I. A. GOULD. Michigan State College.

This study was conducted to ascertain under what conditions and to

what extent rancidity would occur in mixtures of homogenized and unhomogenized raw and pasteurized milk. The lipolytic changes which occurred were determined by titration of the milk and by organoleptic means. In mixtures of unhomogenized raw and homogenized pasteurized milk, acidity increases occurred with as little as one per cent of the unhomogenized milk. Increasing the percentage of the unhomogenized milk in increments up to 50 per cent increased the rate and extent of acidity development, whereas further increases resulted in a decrease in the acidity. The maximum acidity developed over a period of ten days occurred when the ratio of unhomogenized raw milk and homogenized pasteurized milk was 1:1. Rancidity was definitely detected after 7 to 10 days of storage when the mixture contained 5 to 95 per cent of the raw milk or 5 to 95 per cent of the homogenized pasteurized milk.

Mixtures of homogenized raw and homogenized pasteurized milk showed a greater acid development than did the trials in which the raw milk was unhomogenized. Further, the extent of acidity development varied directly with the amount of raw homogenized milk present, with the maximum development resulting in the sample consisting entirely of the raw milk. Development of rancid flavors in general correlated with acidity changes.

The third phase of this study consisting of mixtures of unhomogenized and homogenized raw milk gave results somewhat similar to those secured when mixtures of homogenized raw and homogenized pasteurized milk were used.

The development of rancidity in milk mixtures appears to be equally dependent upon the amount of lipase present and upon the amount of acceleration afforded by the newly created surfaces.

394. Menstruation Frequency and its Relation to Conception in Dairy Cattle. G. W. TRIMBERGER, University of Nebraska, Lincoln, Nebraska.

In two groups of 100 each for heifers and cows not bred at estrus it was found that 100 heifers and 61 cows menstruated. Two similar groups of 100 heifers and 100 cows were bred during estrus. Of the 100 heifers bred at estrus, 81 menstruated, 61 of them conceived and 52 or 85.25 per cent of those conceiving also menstruated, while of the 39 that did not conceive, 29 or 74.36 per cent menstruated. Of the 100 cows bred at estrus, 61 menstruated. Seventy-two of the cows conceived, and 50 or 69.44 per cent of those conceiving also menstruated, while of the 28 that did not conceive, 11 or 39.29 per cent menstruated.

The data do not indicate any definite relationship between breeding and conception as affecting menstruation; contrary to the general opinion among herdsmen, menstruation a few days after service is no indication that conception did not take place. From the total of 303 individuals that men-

struated, 74.26 per cent showed the menstrual discharge on the second day following estrus.

395. Cows' Urine as a Fertilizer for Bluegrass Pastures. W. B. NEVENS, Illinois Agricultural Experiment Station.

Four small plots of Kentucky bluegrass were treated in the spring months with cows' urine at rates ranging from 1250 pounds to 5000 pounds per acre at each application. A control area was untreated. Samples of the grass were harvested monthly from May to September, inclusive.

Although the urine contained more than 1 per cent nitrogen and in most cases more than 1 per cent potassium, the treatments, as a rule, were not harmful to the forage. The protein content of the grass on the treated plots was higher than that of the control area, and in most instances, the heavier the application of urine, the higher the protein content. The yields of the urine-treated plots were higher than that of the untreated pasture and there was a tendency toward higher yields from the more heavily treated plots. The palatability of the grass, as evidenced by close grazing by cattle, was higher on the urine-treated plots and the greater the protein content, the greater the intensity of grazing.

396. Butterfat and Silage Carotenoids. B. CONNOR JOHNSON, W. H. PETERSON AND H. STEENBOCK, Dept. of Biochem., Univ. of Wisconsin, Madison, Wis.

Non-carotene pigments formed by the action of acids in silage are carried over into the butter fat of the milk. By chromatographic separation the distribution of pigments in butters from cows on different forages was found to be as follows: pasture, 80 per cent carotene, 20 per cent non-carotene; molasses alfalfa silage, 68 per cent carotene, 32 per cent non-carotene; phosphoric acid alfalfa silage, 65 per cent carotene, 35 per cent non-carotene.

397. Live Weight of Cow at Various Stages of Lactation in Relation to Milk-energy Yield. W. L. GAINES, Ill. Agr. Exp. Sta., Urbana, Ill.

The problem is approached by dealing with live weight (W) at various stages of lactation and with milk-energy yield for the 8-months partial lactation (FCM). The equation, $FCM = aW^b$ is adjusted to the observations. The exponent, b , varies greatly according to the stage of lactation at which live weight is measured. FCM and W are most closely related where live weight is measured during the first month of lactation ($r = .70$). In general, the evidence of experimental observations is that within a dairy breed and within a herd FCM is proportional to the 1.07 power of live weight, where live weight is measured in the first month of lactation.

398. Effects Which Selection of Dams May Have on Sire Indexes. JAY L. LUSH, H. W. NORTON III AND FLOYD ARNOLD.

The question of what bias is introduced into the proving of sires when

the bull was mated to selected cows was investigated on 676 pairs of daughters and dams used in proving sires in Iowa D.H.I.A. records and on some 3000 pairs used in proving sires in Holstein-Friesian H.I.R. records. Only pairs where the dam had at least two records were used. The mates of each sire were divided into a high half and a low half on the basis of their first record and the regression of their later records on this first record and the regression of their daughters' records on this first record were studied. The selection of dams biases the sire proof by causing the selected dams to have breeding values above the average of the population from which they were taken but lower than the average of the records on which they were selected. This bias affects the difference between daughters and dams most severely. It affects the sire index and the daughter average about equally (the former somewhat more where each dam is represented by only one record) but in opposite directions.

That such a bias is often important in sire proving in actual practice may well be doubted, in view of what has yet been observed about how slightly the intensity of selection varies from herd to herd. The use of lifetime averages automatically tends to reduce sharply the amount of this bias but does not wholly eliminate it. In those individual cases where this bias is thought important, correction for it can be made by correcting the records on which the mates were selected toward their herd average, so as to allow for incomplete repeatability and incomplete heritability. Repeatability of differences in single records within groups of cows mated to the same sire was .43 for fat and .48 for milk in these Iowa D.H.I.A. data and was .40 for fat in the H.I.R. data. Heritability of differences between single records was .28 for fat and .33 for milk in the Iowa data and was .25 for differences between first records and .30 for differences between second records in the H.I.R. data. This is about the same as or a bit smaller than was indicated by earlier published comparisons between the daughters of high record and of low record mates of the same sires. Dominance deviations, epistatic deviations, and permanent effects of environment, all three together accounted for only about 10 to 15 per cent of the variance between records made by the mates of the same bull.

399. The Utilization of Urea by Ruminants as Influenced by the Level of Protein in the Ration. M. I. WEGNER, A. N. BOOTH, G. BOHSTEDT, AND E. B. HART, University of Wisconsin.

A basal ration consisting of corn silage, timothy hay, and a concentrate composed of ground yellow corn and ground oats, half and half (11.3 per cent protein) was fed to a Holstein heifer with a rumen fistula. Varying amounts of linseed oil meal and urea were added to the concentrate to determine the effect of the level of protein in the feed on urea utilization in the rumen. Samples of rumen ingesta were obtained via the fistula and analyzed

for protein and ammonia (urea). The protein content of rumen ingesta showed a decided increase when the level of protein in the concentrate was increased to 24 per cent. By increasing the protein in the concentrate fed to 18 per cent or more the level of protein in the rumen ingesta became greater than 12 per cent. At this point the rate of conversion of urea nitrogen to protein in the rumen began to decrease. When no linseed oil meal was added to the concentrate the added urea was utilized up to a level of 4.5 per cent (protein equivalent of 12 per cent) of the concentrate.

400. The Content of Grass-Juice Factor in Legume Silages and in Milk Produced Therefrom. B. CONNOR JOHNSON, C. A. ELVEHJEM AND W. H. PETERSON, University of Wisconsin.

Seventeen different kinds of silage were assayed for the grass-juice factor by means of their growth-promoting qualities when fed to guinea pigs on a winter milk diet. The acid preserved and soured whey treated silages were found to be somewhat higher in this factor than other types of silage.

The content of grass-juice factor of winter milk was increased by feeding silages rich in the factor, *e.g.*, silages prepared with adequate amounts of phosphoric acid.

401. Effect of Stilbestrol on the Mammary Gland of the Mouse, Rat, Rabbit, and Goat. A. A. LEWIS AND C. W. TURNER, University of Missouri.

Subcutaneous injections of stilbestrol at low dosages caused extensive duct proliferation in male mice in 2 to 4 weeks. Mammary development did not proceed farther in spayed virgin female mice similarly treated. Oral administration of stilbestrol to male mice required approximately five times as high a dosage as by injection to obtain similar results.

Castrate male rats required a higher dosage of stilbestrol than did mice to obtain mammary duct growth.

Four-tenths gamma per day of stilbestrol subcutaneously was adequate to secure extensive mammary duct development in male rabbits. After 40 to 60 days treatment early lobule development was apparent. Percutaneous administration was also effective. Mammary glands from two rabbits with subcutaneous pellets had well developed lobule-alveolar systems and responded well to lactogen treatment at 90 days. Normal females tended to lactate on stilbestrol injection.

Subcutaneous injection of stilbestrol into virgin goats caused abundant and prolonged lactation from lobule-alveolar glands. Little increase in extent of glands was apparent. Subcutaneous administration followed by pellet implantation caused no mammary gland development in a castrate male goat although the teats were hypertrophied.

BOOK REVIEW

402. **Farm Animals, Their Breeding, Growth and Inheritance.** JOHN HAMMOND. 199 pp., 114 fig. Longmans, Green & Co. New York, 1940. \$5.00.

A brief yet reasonably up-to-date and well illustrated account of the physiology of reproduction and growth from the standpoint of what the producer of farm animals or a student just entering this field would find interesting or useful. The first two-thirds of the book is mainly concerned with such topics as time of ovulation, when to breed, artificial insemination, endocrinology as far as it affects these, differences in ages at which various parts of the body grow most rapidly, market requirements concerning weights and bodily proportions, etc. There is a separate chapter for each kind of farm livestock. The latter third of the book deals with Mendelism, evolution, selection, breeding systems and special problems in breeding for productiveness in the various kinds of livestock. J.L.L.

BACTERIOLOGY

403. **Classification of the Organisms Important in Dairy Products. III. *Pseudomonas putrefaciens*.** H. F. LONG AND B. W. HAMMER. Iowa Agr. Exp. Sta. Res. Bull. 285. Jan., 1941.

A technique for isolation of *Ps. putrefaciens* from water, butter and creamery equipment is described. The following special medium was developed to aid in the isolation: gelatin—4.0; proteose peptone—2.0; dipotassium phosphate—0.1; ferric ammonium citrate—0.05; agar—1.5 per cent; and water to make 100 per cent. *Ps. putrefaciens* developed readily on this medium and after several days colonies were fairly large, raised and brown to reddish brown or pink.

In certain cases enrichment in litmus milk at 3° C. followed by smearing on the special medium aided in isolation while with other materials direct smears on the medium were more frequently successful. Smears prepared with the serum of butter were more successful than smearing the butter itself. Enrichment in laboratory churnings of butter held at 3° C. also aided in certain isolations.

Ps. putrefaciens was isolated from raw and pasteurized milk, raw sweet cream, numerous samples of butter, stream, lake and roadside water in different parts of the country, private well water, city water supplies, moist soil, floors, sewers, creamery floors, churns, and various other pieces of dairy plant equipment.

Heat, acid and salt easily destroy *Ps. putrefaciens*. Pasteurization by either the flash or holder methods of pasteurization should kill any organisms of this type that might be present in the cream. Sour cream and highly ripened unsalted butter failed to yield the organism. Of 15 representative

cultures all grew in litmus milk containing 10 per cent salt, 6 in 6 per cent, 1 in 8 per cent, and none in 10 per cent salt.

In a total of 176 cultures studied in detail, variations occurred but the authors considered these too insignificant to justify varietal differences. From the standpoint of identification, the outstanding characters of the organism were found to be action on litmus milk, morphology, phosphatase production, and action on butter.

P.R.E.

CHEESE

404. **Western Hemisphere Cheeses Replace European Imports.** ALLEN CHAFFEE, New York, N. Y. *Food Indust.*, 13: 3; 58-60, 107-108. 1941.

In November 1939, we imported 6,343,578 pounds of cheese as compared with 2,260,737 pounds in November 1940. Of the 1940 imports, a large amount were Argentine imitations of European types.

Because of the war, imports have stopped or nearly so, on the following cheeses: French Roquefort, all of the Italian hard-grating cheeses such as Parmesan, English Stilton, Netherlands Edam and Gouda, Danish Blue, and some of the Scandinavian goat's milk cheeses such as Gjetost.

Argentine and U. S. manufacturers are working on imitations and substitutes for the French Roquefort and Danish Blue cheeses. Opinions differ as to the quality of these U. S. and Argentine cheeses, but the manufacturers are taking it seriously and great steps forward are being made.

U. S. manufacturers have made a few substitutes for the Italian hard-grating cheeses and Argentina has made imitation Parmesan and other hard-grating cheeses. These Argentine cheeses have not been of good quality in the opinion of many importers but are improving.

Edam and Baby Gouda cheeses are past the experimental stage in the U. S. and are readily accepted by the American housewife. Methods for making trappist type cheese have been standardized but commercial production is still small.

Some of the Scandinavian cheeses are being made in the U. S. Some Swiss cheese is still being imported and American made Swiss cheese is meeting with some success.

J.C.M.

405. **The Keeping Qualities of Cheese and Other Milk Products.** H. D. MURRAY AND A. TYRRELL. *Food Mfr.*, 16: 2; 39-40. 1941.

In this article the authors first give the methods used to render the milk stable and then review the production and uses of the various milk products, especially cheese, from the standpoint of keeping quality.

Milk may be rendered more stable (1) by dehydrating without altering the proportion of solids originally present, (2) by withdrawing water and

other constituents so as to yield an altered product which can be stored without elaborate precautions. In the first class are evaporated, dried and condensed whole and skimmilk, and in the second class are cream, butter and cheese.

Cheese may be made by precipitation of the curd by rennet or by acid-producing bacteria. Where rennet is used the pH is considerably higher and this results in a saving in the cheese of a greater proportion of the insoluble salts, and renders the curd capable of supporting a larger variety of micro-organisms. This last makes possible production of a greater variety of flavors, but it also makes the cheese more liable to spoilage.

A new development in the keeping of cheese is the pasteurization of the ripened cheese forming processed cheese in which micro-organism activity is almost at a standstill.

In all phases of cheese manufacture the pH is of great importance in determining the keeping quality of the product. J.C.M.

406. **Bacteriology of Cheese. V. Defects of Blue (Roquefort-Type) Cheese.** H. W. BRYANT AND B. W. HAMMER. Iowa Agr. Exp. Sta. Res. Bull. 283. Oct., 1940.

Black discoloration and a musty flavor in blue cheese were attributed to the growth of a mold, *Hormodendrum olivaceum*. Most of the spoilage occurred in the punch holes and cracks in the cheese. Blue cheese and cheddar cheese were made from milk inoculated with *Aerobacter aerogenes*, the organism commonly responsible for gassy defects in cheddar cheese. Only insignificant numbers of gas holes developed in the blue cheese while the cheddar cheese developed numerous gas holes. The inability of *Aerobacter aerogenes* to produce the gassy defect in blue cheese was attributed to the open texture which permitted the developed gas to escape and also to the unfavorable conditions for growth of the gas-forming organisms in the cheese.

Excessive moisture in certain parts of the cheese was shown to result in a defect characterized by a pronounced softening of the edges.

An outbreak of a defect characterized by a lack of mold growth in blue cheese was investigated. The defect was attributed to use of an atypical strain of *Penicillium roqueforti*.

An attempt was made to determine the cause of gray discoloration, a common defect of blue cheese. In cheese showing this discoloration a mousy, ammoniacal odor commonly developed and the cheese tended to become soapy with age. Two possibilities suggested for the cause of this defect were extensive growth of *P. roqueforti* and the presence of contaminating organisms. P.R.E.

407. **The Cheese Industry in War-Time.** JOHN G. DAVIS. Food Mfr., 15: 8; 204-206. 1940.

This is an article discussing the making of cheese in England during this war.

It is pointed out that no better utilization of surplus milk is possible than the making of cheese, providing the whey is economically used.

The use of whey in preserving silage, as a source of lactose and for beverages are some of the ways pointed out by which nearly all the food value of the whey may be obtained.

J.C.M.

DISEASE

408. **Milking Machines—Their Operation and Effect Upon Milk Production and Mastitis.** A. C. DAHLBERG. N. Y. Agr. Exp. Sta., Geneva, N. Y. The Assoc. Bull., Internat. Assoc. Milk Dealers, 33rd yr. No. 13; 336-340. 1941.

When cows were milked by machine in 9 to 10 minutes or in 5 minutes milk production was less persistent than when hand milking was employed. In a lactation period the final month's production in the case of hand milking was 66.7 per cent of the first month; machine milking in 9 to 10 minutes was 49.4 per cent; and machine milking in 4 to 5 minutes was 52.8 per cent. Production started to decline earlier in the case of longtime machine milking as compared with short time machine milking. It is also concluded that faster milking results in less mastitis. Favorable results were obtained by milking on a definite time schedule with a certain number of minutes for the milking period.

E.F.G.

409. **Disease Dissemination Through Public Sales.** R. A. HENDERSHOTT, New Jersey Dept. Agr., Trenton, N. J. The Assoc. Bull., Internat. Assoc. Milk Dealers, 33rd yr., No. 13; 331-335. Feb., 1941.

The great importance of the public sale as a factor in the spread of animal diseases and the lack of sanitary supervision at sales is cited. The details of the operation of a market where qualified veterinarians under the State Veterinarian examined livestock and certified as to their health are described. The author recommends that all public sales be placed under the supervision of the agency in that state which is charged with the control of diseases of livestock. Rules are suggested which would prevent diseased animals being sold for purposes other than slaughter and going back on the farm to spread disease.

E.F.G.

FEEDS AND FEEDING

410. **Yield, Chemical Composition and Feeding Value for Milk Production of Alfalfa Hay Cut at Three Stages of Maturity.** J. R. DAWSON, D. V. KOPLAND AND R. R. GRAVES. U. S. D. A. Tech. Bull., 739. Oct., 1940.

One each of three fields for three years was cut in the 10 per cent bloom, 50 per cent bloom and full bloom stages. In addition to total yields, chemical analysis and effect upon the stand, the feed value for milk production was determined by feeding each class of hay to three cows receiving the hay only.

There was no observable difference upon the stand. The total yield expressed as 100 per cent for the 10 per cent bloom stage was 99.4 per cent for the 50 per cent bloom and 77.7 per cent for the full bloom. The crude protein content was 18.24, 18.29 and 15.71 per cent, and the total protein per acre was 1,427, 1,381, and 977 pounds, respectively, for the three stages of cutting.

The crude fiber content was 28.86, 28.45 and 32.68 per cent, respectively, for the cuttings. T. D. N. yield per acre expressed in terms of 100 per cent for the 10 per cent bloom stage was 94.7 per cent and 70.2 per cent, respectively, for the half-bloom and full-bloom stages. The average digestion coefficients for crude fiber for the three stages were 47.7, 41.4 and 38.3 per cent, respectively. For crude protein these figures are 77.7, 77.1 and 75.4.

The daily consumption of hay was about the same, 39.4 pounds for 10 per cent bloom, 39.6 pounds for 50 per cent bloom and 38.5 pounds for full-bloom hay.

The nutrients in the earlier cut hay seemed more efficient for milk production. The calculated yield per acre of 4 per cent fat corrected milk was 6,330 pounds from the 10 per cent bloom, 5,254 pounds from the 50 per cent bloom and 3,970 pounds from the full-bloom stage. The cost per ton of hay was \$7.27, \$7.40 and \$8.53, respectively. The calculated cost per 100 pounds, 4 per cent fat corrected milk was \$.51, \$.62 and \$.74, respectively, for the three hays and the costs per 100 pounds of T. D. N. were \$.696, \$.767 and \$.906.

W.E.P.

411. Studies on Riboflavin and Thiamin in the Rumen Content of Cattle.

C. H. HUNT, C. H. KICK, E. W. BURROUGHS, R. M. BETHKE, A. F. SCHALK AND P. GERLAUGH, Ohio Agr. Exp. Sta., Wooster, Ohio.
J. Nutr. 21: 85-92. 1941.

Bioassays of the rations and rumen contents of steers fed various rations were made for riboflavin and thiamin. The results indicated that riboflavin was synthesized in the rumen of steers fed a ration of yellow corn, alfalfa hay and a protein supplement.

The ingesta of a steer fed alfalfa alone contained less riboflavin than did the hay. The data also indicate the synthesis of thiamin in the rumen of steers.

C.F.H.

412. A Quantitative Study of Vitamins in the Ruman Content of Sheep and Cows Fed Vitamin-low Diets. III. Thiamin. L. W. Mc-

ELROY AND H. GOSS, Div. Animal Husbandry, Univ. of California, Davis, Calif. *J. Nutr.*, 10: 163-173. 1941.

Four sheep were fed a ration containing less than 0.4 microgram of thiamin per gram. The dried rumen and reticulum contents of the sheep contained approximately 7 micrograms of thiamin per gram.

Thiamin was detected in the rumen contents of a non-fistulated cow fed a ration deficient in this aspect but not in the rumen-content of two fistulated cows. The authors conclude that their data confirm the work of other investigators that thiamin is not a dietary essential for ruminants.

C.F.H.

413. **Carotene and Vitamin A in Cattle Blood Plasma with Observations on Reproduction Performance at Restricted Levels of Carotene Intake.** R. E. DAVIS AND L. L. MADSEN, Bureau of Animal Industry, U. S. D. A. Washington, D. C. *J. Nutr.*, 21: 135-146. 1941.

The carotene and vitamin A content of the blood plasma of beef cattle was determined spectrophotometrically. The method for determination of vitamin A gave relative values because of substances in the plasma extract which cause some interfering absorption.

The animals were depleted of their reserve carotene and vitamin A before breeding. The authors concluded that vitamin A content of the blood plasma is closely related to the carotene content. Two heifers that received 60 micrograms of carotene per kilogram of body weight from dehydrated alfalfa leaf meal previous to and throughout the gestation period gave birth to apparently normal calves. Cows receiving 30 and 45 mg. of carotene produced abnormal calves.

C.F.H.

FOOD VALUE OF DAIRY PRODUCTS

414. **Physicochemical Assay of Vitamin A.** NORRIS DEAN EMBREE, Distillation Products, Inc., Rochester, N. Y. *J. Indust. Engin. Chem., Analyt. Ed.*, 13: 3; 144-145. 1941.

Data are given on the stability of vitamin A when exposed in clear and brown glass bottles to daylight. Various fish liver oils and vitamin A concentrates when exposed for 5 hours in clear glass lost from 10 per cent to 55 per cent of their potency while the samples exposed to daylight in brown glass lost from 0 per cent to 7 per cent potency. The author recommends the use of brown laboratory glassware by those engaged in performing vitamin A assays on food, medicinal, and physiological preparations.

B.H.W.

415. **Determination of Total Vitamin A Content of Dairy Butters. Spectrophotometric Method.** R. H. NEAL, C. H. HAURAND AND F. H.

LUCKMAN, The Best Foods, Inc., Bayonne, N. J. J. Indust. Engin. Chem., Analyt. Ed., 13: 3; 150-154. 1941.

A relatively rapid spectrophotometric method for the determination of total vitamin A content (vitamin A plus carotene) of butter is described. The fat is removed from melted butter and saponified in a solution of KOH in alcohol. The unsaponifiable matter is extracted, concentrated, and dissolved in cyclohexane. Part of the cyclohexane solution is irradiated for 1 to 1½ hours to destroy its vitamin A and carotene. Using the irradiated portion as a control, vitamin A and carotene are then determined simultaneously in the unsaponifiable extract by means of the spectrophotometer. The method was shown to yield results that were in satisfactory agreement with themselves and in reasonable agreement with the U. S. P. XI method.
B.H.W.

416. **The Calorie and Our Dairy Products.** F. H. PLETCHER. Borden Farm Products, Brooklyn, N. Y. Milk Dealer, 30: 5; 31, 82-85. 1941.

This article is a brief history of early research in nutrition followed by an explanation of how the caloric value of foods is determined. C.J.B.

ICE CREAM

417. **Detection of Sodium Alginate in Dairy Products.** CHARLES W. SCHROEDER AND PHILEAS A. RACICOT, Mass. Dept. Public Health, Boston, Mass. J. Indust. Engin. Chem., Analyt. Ed., 13: 3; 165-166. 1941.

Since commercial sodium alginate generally contains dextrin, a quick preliminary test for dextrin may be used to establish the probable presence of alginic acid in the dairy product. To test for alginic acid, add to the sample a volume of hydrochloric acid equal to the water content of the dairy product, boil, centrifuge and repeatedly wash the solids removed by centrifuging. Dissolve as much of this residue as possible in NaOH, filter and add to the filtrate an equal volume of 95 per cent alcohol. Centrifuge and wash the solids with 75 per cent alcohol. Dry the gum so obtained and dissolve as far as possible in 0.1 N NaOH, add H₂SO₄ and let stand. If a color develops, it indicates the presence of alginic acid. By this test it is possible to detect the presence of 0.2 per cent or more of sodium alginate.

B.H.W.

418. **Taste, the Secret of Fountain Success.** ROBERT L. SWAIN, Editor, "Drug Topics," New York. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., 4: 22. Oct., 1940.

"Taste—the Secret of Fountain Success," is the slogan used in a campaign to increase profits among the retail druggists of the country. Taste appeal should gratify all 5 senses and thus create the most favorable and lasting impression on the customer.

A survey of retail drug stores revealed that installation of a new soda fountain increased fountain business 21.7 per cent immediately; the business for the store as a whole immediately increased 14.4 per cent and eventually 20.9 per cent. The conclusion has been reached that an untapped volume of soda fountain business is available to the aggressive merchandiser.

M.J.M.

419. **Working with Your Dealers.** F. N. RUSSELL, Russell Creamery Co., Brainerd, Minn. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., 4: 46. Oct., 1940.

Ice cream sales can be increased by getting more accounts or by building up our present accounts. The industry is urged to spend less effort on the first and more on the second method. The advent of stores selling ice cream exclusively has shown that much more gallonage can be sold through an outlet than has been thought possible in the past. The per capita consumption of ice cream has doubled during the past 20 years but is still sufficiently low so that further sizable increases are possible.

By modernizing stores and fixtures, by planning so that the ice cream department predominates in the store, and by advertising ice cream rather than brand names, ice cream sales through present outlets can be increased.

M.J.M.

420. **Merchandise and Survive.** B. J. LOFTUS, Abbotts Dairies, Inc., Philadelphia, Pa. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., 4: 52. Oct., 1940.

The ultimate aim of all merchandising is to stimulate sales so that your company and the dealer will both profit. The dealers are waiting for help and the ice cream manufacturer need not keep them waiting. The author of this article advises the ice cream industry to use the material made available by the merchandising council of the Ice Cream Merchandising Institute. By making this material available to dealers an effective merchandising campaign should be the result.

M.J.M.

421. **Dextrose and Corn Syrup for Frozen Desserts.** A. C. DAHLBERG AND E. S. PENOZEK, N. Y. Agr. Exp. Sta., Geneva, N. Y. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., 2: 31. Oct., 1940.

Three different types of corn sweeteners, dextrose, enzyme-converted corn syrup and corn syrup solids, were used in this study. The amount of sucrose

that may be replaced in ices and sherbets and ice cream was found to be about 25 per cent for best results in flavor, body and texture.

The authors state that the same relative sweetness in ice cream was secured when one pound of sucrose was replaced on a dry solids basis with 1.5 pounds of enzyme-converted corn syrup, 2 pounds of corn syrup solids and 1.1 pounds of dextrose. When 25 per cent of the sucrose was replaced on this basis, the hardness and melting rates were affected only slightly. The smoothness and richness of the ice creams were improved by the corn syrups and consumer preference slightly favored them.

A replacement of sucrose pound for pound by corn sweeteners does not give equal sweetness in ice cream, but a finished product may result which is perfectly satisfactory to the trade. The character of the sweetness is described as being different between sucrose and dextrose, with the former giving a more intense effect of shorter duration in the mouth. The question is also raised as to the desired sweetness of ice cream. During the past 20 years the sugar content of ice cream has been increased; at present most ice creams are between 14 and 16 per cent sugar. A lack of a specific percentage of sugar which satisfies most customers, and the desirability of varying the sugar content with the geographic location and the character of the ice cream itself, complicates the problem of relative sweetness of ice cream from a commercial view point.

M.J.M.

422. **The Application of Motion Pictures as a Medium in Showing the Influence of Several Factors Upon the Stability and Melt-Down Properties of Several Different Kinds of Ice Cream.** W. H. E. REID, University of Missouri. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., 2: 101. Oct. 1940.

The application of motion picture photography has made it possible to show the relationship of several factors in the composition and manufacturing of ice cream which influence the stability and melting appearance of ice cream. These studies have been made with both black and white and colored films. The effect of a number of factors upon the melting characteristics have been determined by photographic means.

M.J.M.

423. **Creative Selling.** DAVE COLCORD, Haskell, Oberlin Co., Marengo, Ill. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., 4: 11. 1940.

Only a small percentage of the market for ice cream has been developed. The big volume of sales lies in territories which are not using enough ice cream. Since this is so, the combined efforts of ice cream salesmen should mean more business for everybody. Time and energy do not have to be spent in trying to get competitor's accounts, but in developing new business.

The job of the salesmanager is to teach those under his direction how

to create new business. This paper is a discussion of various ways to accomplish this objective.

M.J.M.

424. **The Control of Overrun in Ice Cream.** H. C. THOMAS, Harrington & Co., Dushare, Pa. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., 3: 39. Oct., 1940.

The ice cream consuming public and retail dealers are becoming increasingly conscious of the weight of ice cream. The production department must satisfy the salesmen and dealers by furnishing ice cream which is uniform in weight from can to can. When this is so, then a salesman can show the retailer exactly what the margin of profits will be for each can of ice cream which is dispensed.

With batch machines, overrun was controlled within reasonable limits by the use of overrun scales. When continuous freezers were installed it was then possible to control the weight of each package at the freezer. To aid in this control, scales are placed at freezers and packaging machines and the cans and packages are carefully checked as they are filled.

Reports are kept at the freezer of the unit weight of each flavor made, the number of units made, and the amounts of mix and flavorings used. Except for some shrinkage, the weights of materials used and of the finished ice cream should check. These reports, which are tabulated on monthly summary sheets, give a good record for the study and control of plant production.

M.J.M.

425. **Flavor Costs.** J. E. SHIPLEY, Abbotts Dairies, Inc., Philadelphia, Pa. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., 3: 36. 1940.

With the exception of dairy products, the cost of flavoring materials is the largest single item in the material cost. The cost of flavoring ice cream varies from 10 per cent to 40 per cent of the total material cost, depending on the kind of flavor and quantity used.

Examples are given for arriving at the cost of 5 different ice creams.

One can change the cost of any flavor by changing any one of the following items. 1. Quantity of fruit or flavor used. 2. The percentage of butterfat in the ice cream. 3. The amount of overrun.

Public acceptance and legal standards set the minimum on flavors costs, but there are no limits set for the cost of the mix and flavoring. With this in mind it should be well worth while to check the costs on all new flavors before they are placed on sale. In this manner the introduction of an unprofitable item can be avoided.

M.J.M.

426. **What the Association Expense Comparisons Have Meant to Us.** F. SCHWARTZ, Dairymen's League Coop. Assoc., Inc., New York,

N. Y. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., 3: 33. Oct., 1940.

The benefits which can be derived from participation in the annual expense comparisons conducted by the International Association of Ice Cream Manufacturers are discussed. The comparisons furnish a yardstick for measuring plant efficiency, distribution costs, and so on. A basis for budgeting for advertising is furnished. The comparisons can serve as a means of arriving at unit costs, and the management learns whether unit expenses for manufacturing and distribution are in line with the expenses of other companies. M.J.M.

427. **Catching Up With Employee Frauds.** J. S. SEIDMAN, Seidman and Seidman, New York, N. Y. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., 3: 17. Oct., 1940.

Over 200 million dollars a year are lost to industry through employee frauds. This, however, is only the amount known to be lost and does not include undetected frauds. Frequently auditing does not reveal the frauds, but they are discovered accidentally.

The nature of fraud and what can be done to eliminate it are the points stressed in this discussion. Auditing has shown sufficient plasticity and progress to justify the statement that by careful audits fraud can gradually be eliminated.

A classified outline of types of fraud reported in case studies is included in this paper. M.J.M.

428. **A New Association Accounting System.** O'NEAL M. JOHNSON, Statistical and Accounting Bureau, I.A.I.C.M., Washington, D. C. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., 3: 11. Oct., 1940.

The accounting system for ice cream plants, which has been developed by the statistical and accounting bureau of the International Association of Ice Cream Manufacturers, has been revised in some respects. This has been necessary because of such changes as the advent of the continuous freezer, which has eliminated much packaging machinery, newer methods of pro-rata of service costs, more knowledge about dealer costs, and so on. The necessary changes and the suitability of the new system to meet these changed accounting conditions is explained. M.J.M.

429. **Transport Refrigeration.** JOHN H. REILLY. Refrig. Engin., 41: 3; 167. 1941.

A brief description of transport refrigeration development and details of hold-over plates application. The latent heat value of the eutectic solu-

tion in the hold-over plate is approximately 106 B. T. U. per pound. The freezing point of the eutectic for low temperature work is about -6° F. and for high temperature work approximately 18° F.

In figuring the refrigeration load for hold-over plates, two factors must be considered: (1) the necessary amount of plate surface must be used to offset the heat leakage and service load; and (2) it is necessary to have sufficient hold-over solution to absorb the heat gained in the number of hours the truck is on the route, or the length of time hold-over is desired.

Vacuumized hold-over plates result in freedom from bulging when eutectic solution is frozen and causes plates to press against coils more closely. Plates may be installed horizontally at ceiling of body, vertically on ends and sides, or may be used as partition walls. L.M.D.

430. New Developments in Ice Cream Stabilizers. B. I. MASUROVSKY.
Ice Cream Trade J., 37: 2; 37. 1941.

Domestic production of gelatine has been stimulated since the war started. Should there be a shortage of gelatine as a result of decreased importation from Europe, ice cream manufacturers will have available several other colloidal products for use as stabilizers. Among the newer types of stabilizers that are now available, are patented milk products which have some of the milk sugar removed, pectin derived from citrus fruits, cereal products of a glutenous nature, and stabilizers made from seaweeds such as kelp, and that made from other marine products such as Chondrus Crispus (Irish moss). W.H.M.

431. Streamlined Merchandising as the Dealer Will Accept It. LOUIS J. WAINER, Penn Dairies, Inc., Lancaster, Pa. Ice Cream Trade J., 37: 2; 32. 1941.

An ice cream merchandising plan which dealers will accept must be planned carefully, followed aggressively, be simple and easy for the dealer to follow. Once such a program is put into action, consistent follow up action is needed. The effort which it takes to increase the volume of business done by a dealer is usually less than that required to get a customer away from a competitor and usually much less expensive. A survey of the locality around a dealer's store, to show him the potential volume of business which he should have, suggestions on how to improve the appearance and cleanliness of his store, and the use of the proper equipment and methods for serving ice cream, were cited as examples of things which a manufacturer might do for his dealers. W.H.M.

432. Ice Cream Production. H. BAYER, General Ice Cream Corp., Schenectady, N. Y. Ice Cream Trade J., 37: 2; 18. 1941.

The importance of good housekeeping and sanitation are sometimes overlooked by the ice cream plant production managers, yet they are both important in the production of a quality product. A good production man must have some training in cost accounting and know when and how to purchase raw materials, how to plan production so that the best use may be made of labor. The use of motion pictures which show the various plant operations was suggested as a means of locating lost motion in plant operations. Other factors which must be watched closely by the production manager if costs are to be held down, are: securing a satisfactory yield on ice cream and novelties, efficient operation of refrigeration machines, and the avoiding of damage to equipment and possibility of accidents that may result from the use of too much water on floors.

W.H.M.

433. **The Manufacture of Coffee Ice Cream.** B. I. MASUROVSKY. *Ice Cream Trade J.*, 37: 3; 51. 1941.

A brief history of the origin and source of the supply of coffee, and the composition of raw and roasted coffee is given. In testing coffee extracts to be used in ice cream the hot water test can be used. In making the test eight ounces of boiling water and 1 ounce of coffee flavoring are mixed in a glass container. The mixture is smelled and tasted and any objectional features noted. Observation on the clarity of the mixture can be made. It requires about one and one half times more coffee flavor for sherbets than for ice cream.

W.H.M.

434. **Menu Merchandising.** ROBERT J. COOLEY. *Ice Cream Trade J.*, 37: 3; 16. 1941.

The importance of an attractive menu in the merchandising of ice cream is discussed. A retail store menu card successfully used by Williams—McWilliams Dairy Products, Inc., Ft. Lauderdale, Fla., is described in detail. The menu raised the gross margin of the fountain and luncheonette from 37.5 to 52 per cent by increased prices which were successfully merchandised to the consumers by means of the colorful invigorating, brilliant, oversized menu. Menus may be supplemented with printed or lettered wall signs. A large New Orleans store uses 18 x 24 inch colorful signs within frames for interior store walls. A billboard, erected on the store's parking lot may be used to call attention to some of the outstanding values on the menu.

W.H.M.

435. **Fitting Ice Cream in the Food and Drug Act.** CHARLES M. FISTERE, Dairy Industries Committee, Washington, D. C. *Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs.*, 2: 94. Oct., 1940.

A proposed regulation for foods represented for special dietary use

would put these foods, as far as label declarations are concerned, under the Food and Drug Administration. A number of regulations proposed would bring hardship to the dairy industry. It is the plan of the industry to attempt to secure clarification, or relief if necessary, when the hearing is resumed, by asking that the law be construed as not including dairy products in the category of special dietary foods.

The industry's proposal provides for a standard of identity for ice cream mix, a definition for vanilla ice cream, chocolate ice cream, fruit ice cream, nut ice cream, and finally, for ice cream containing other flavoring materials. The proposal includes a suggested definition for sherbets and for ices, thereby covering the field.

M.J.M.

436. **The Injection of Fruit and Syrups in Frozen Ice Cream.** A. C. ROUTH, Esmond Dairy Co., Sandusky, Ohio. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., 2: 90. Oct., 1940.

After experimenting with a large number of injecting devices the following method was perfected. The method consists of metering a predetermined quantity of flavoring, by a positive pump of variable speed to the extrusion point. The next problem was to devise a means of combining the flavoring material with the ice cream. From this work a large variety of combinations has been worked out. Types of syrups and fruit mixtures which will not "bleed" out or form a surface crust were developed.

With the above experience as a background, variegated ice cream has been offered to the industry, under the trade name "Revel." Marketing experience of the first year indicates that this basic development presents an added responsibility to the benefiting industry.

M.J.M.

437. **Handling Labor in the Plant.** LOUIS G. GALLIKER, Galliker Ice Cream Co., Johnstown, Pa. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., 2: 87. Oct., 1940.

Approximately 53 per cent of our total yearly gallonage of ice cream is made in the 5 summer months. When the employees were paid by the hour, monthly pay checks were highest in the summer when living costs were low and were lowest when living costs increased. Consequently the help was dissatisfied.

To overcome the difficulties a salary program was worked out for the plant men. The average number of hours of work during the previous year was taken as a base. The yearly salary was calculated on the basis of the number of hours and hourly rate. The men were given a yearly increase which was added to the above amount. The total figure divided by 24 gave the amount of the semi-monthly salary check. In the winter time when work is less than 40 hours a week, the difference is carried over and made

up during the summer time. Each employee receives a week's vacation with pay. When a man works after 7 p.m. he gets paid for overtime.

The employees are well satisfied under the new plan because it assures them a constant income during the winter months. M.J.M.

438. **Dollars and Sense in Plant Sanitation.** FRED E. YETZ, The Borden Co., New York, N. Y. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., 2: 67. Oct., 1940.

On the average the present day frozen desserts plant maintains a high degree of sanitation. It has been reported from reliable sources that the industry has progressed to the point of surpassing the milk industry in many respects.

Sanitary practices have changed materially, and a corresponding change in costs has occurred. The author believes that in many instances the added costs due to sanitary measures are not justified. An example is cited of a compact set-up of 4 freezers, three fruit injectors, and incidental piping. When all this equipment has been used it is necessary to dismantle, clean, and assemble 459 parts, 81 nuts, and 19 screws. Due to the complexity of modern machines, it has become necessary to use a higher type of employee on clean-up work than was the case some years ago. This inadvertently constituted a wise move because maintenance and replacement costs have thus been reduced.

A committee of Sanitary Control has been created within the Association. This committee is working for the adoption of reasonable and uniform standards for dairy products moving in interstate commerce. This should eliminate unnecessary expense due to lack of uniformity of requirements for inspection of methods and equipment, involving both producers and manufacturers of dairy products.

In conclusion, it was stated that a program of unquestionable and efficient sanitation calls for the allotment of a certain fixed amount of the manufacturing expense for the accomplishment of this phase of the work.

M.J.M.

439. **What's New in the Dairy Industries Exposition.** R. E. WRIGHT, Jersey Dairy Products Corp., Baltimore, Md. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., 2: 64. Oct., 1940.

New developments in several types of equipment were stressed, such as more efficient freezers and fruit feeders, flake ice machines, homogenizers, cup fillers and bar machines, stainless steel fittings, and delivery equipment. Sanitary construction and greater efficiency are the outstanding points of development in the new machinery specifically mentioned in the discussion.

M.J.M.

440. Factors Affecting the Viscosity and Coverage Value of Chocolate Coating for Ice Cream. J. HOFFMAN ERB, Ohio State University, Columbus, Ohio. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., 2: 52. Oct., 1940.

A number of factors determine the coverage results which will be obtained with a coating. These are as follows: 1. Composition (especially fat) of the coating. 2. Fineness of grinding. 3. Heat treatment when melting. 4. Temperature of the coating, as well as the ice cream, during the dipping operation. 5. Amount of moisture incorporated into the melted chocolate at the dipping tank. 6. The use of lecithin.

An ideal dipping arrangement was found to be somewhat as follows:

The bars were hardened in a brine tank, taken from the moulds, and dried thoroughly by chilling before dipping. The coating was melted and held in a large jacketed vat with a lid. The temperature was maintained at 115° F. by a thermostat. The coating was constantly circulated to the dipping tank and back to the vat. The viscosity of the chocolate did not increase during the dipping operation, under the above conditions.

A desirable test for controlling dipping operations is the bob test. The principle of the test is to dip a carefully weighed metal bob into the chocolate, allow it to drain and determine by weight the amount of chocolate remaining on the bob. The bob test is of great value in determining the suitability of new coatings of unknown composition. It is also very useful as a plant control test. By running bob tests at intervals, variations in the thickness of the coating due to changes in temperature, absorption of water, etc., can be detected and remedial measures can be made. Thickening of the coating due to moisture absorption is common. This can be prevented by pre-chilling the bars, which gives a better adherence between the coating and the ice cream. Coating remaining in the dipping tank from a previous day's run usually contains moisture and new coating should not be added to it.

M.J.M.

441. Report of the Committee on National Standards for Ice Cream Brick Molds and Cartons. RIDGWAY KENNEDY, JR., Abbotts Dairies, Inc., Philadelphia, Pa. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., 2: 51. Oct., 1940.

Standard dimensions for ice cream brick molds and cartons were promulgated in 1936 and accepted by the industry. These standards have been found to be still satisfactory and no further changes are necessary.

The committee has studied the dimensions desirable for cartons to be used for storing ice cream in the compartments of household refrigerators. The division of simplified practice of the National Bureau of Standards has been advised of these dimensions for the carton, which have been added to

the list of U. S. Standards for Ice Cream Brick Molds and Cartons. Ice cream manufacturers have been advised as to the dimensions of this carton.
M.J.M.

442. Use of Enzyme Converted Corn Syrup in the Manufacture of Ice Cream, Sherbets and Ices. P. H. TRACY, University of Illinois, Urbana, Ill. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., 2: 47. Oct., 1940.

The recent introduction of enzyme converted corn syrup has made it desirable to study the merits of this product in ice cream, sherbets and ices. In comparison with sucrose, it depresses the freezing point of mixes somewhat more, but it has less effect in this respect than does corn sugar. It is possible to replace about one-third the sucrose with enzyme converted corn syrup, whereas, with corn sugar the replacement is usually limited to one-fourth. A one-third replacement causes a slight increase in mix viscosity and somewhat lowers the whipping ability of the mix; the flavor is unaffected but the body of ice cream is improved by the replacement.

The author states that in ices and sherbets replacements of sucrose up to 50 per cent by enzyme converted corn syrup are satisfactory. The replacements improve the body and flavor of the ices and sherbets and aid in controlling "bleeding" and surface crustation.
M.J.M.

443. Vacreation of Ice Cream Mix. N. E. FABRICIUS, Iowa State College, Ames, Iowa. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., 2: 23. Oct., 1940.

The practice of condensing ice cream mix in a vacuum pan has been followed for several years by some manufacturers. This procedure is believed to improve the flavor of ice cream because some off-flavors are drawn off with the vapors from vacuum treated mix. This paper presents studies made with a system of steam diffusion with vacuum treatment of ice cream mix. The machine used is called the "Vacreator" and was invented by H. Lamont Murray of Auckland, New Zealand.

In 19 comparisons of vacreated mix with vat-pasteurized ice cream mix of the same composition, an improvement in flavor score of 1.29 points was secured in the vacreated product. The treatment improves the flavor of fresh ice cream mix and the improvement is more evident the longer the ice creams are held in storage.

Vacreations of ice cream mix delayed the development of such off-flavors as stale, oxidized or metallic in the finished ice cream. The improvements in flavor score as a result of vacreation were greatest during the seasons when the fresh cream was slightly defective in flavor. This was especially true during early summer when the cream was grassy in flavor and slightly acid, and in winter when the cream was occasionally slightly rancid.

The removal of off-flavors in the ice cream made it possible to use less flavoring material and still obtain a distinctive flavor.

When using direct steam in pasteurization, it was found necessary to use water and water softeners which do not impart off-flavors to the steam. Off-flavors in the steam would be transmitted to the ice cream mix.

M.J.M.

444. The Effect of Certain Factors on the Keeping Quality of Frozen Cream. C. D. DAHLE, R. K. LAWHORN AND J. L. BARNHART, Pennsylvania State College, State College, Pa. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., 2: 7. Oct., 1940.

The main purpose of the experimental work reported in this paper was to study factors affecting the keeping quality of frozen cream containing 40 per cent fat. The cream was held for 6 to 9 months before being used in ice cream as the sole source of butterfat. From their experiments the authors draw the following conclusions:

1. The oxidation-reduction potential of cream was lowered by heat above 170° F. and was increased by copper. The Eh determined during storage is not a reliable index of the keeping quality of cream, since the Eh during storage increased greatly during the first month and then decreased to a point usually lower than the initial reading.

2. The initial Eh reading of the pasteurized cream before storage is a fair index of the keeping quality, though not entirely reliable. It was found that cream with added copper having an initial Eh of above 0.30 volts usually developed an oxidized flavor during storage of 6 to 8 months, while those below 0.30 volts seldom did. There were a few exceptions, however.

3. Cream with a cooked flavor was slower to develop an oxidized flavor. These creams had an Eh below 0.30 volts.

4. The higher temperature pasteurization (170–190° F.) resulted in creams of superior keeping quality when copper was present in abnormal amounts.

5. A pasteurizing temperature of 150° F. for 30 minutes offered no protection during storage if the copper content exceeded 0.6 p.p.m. A temperature of 170° F. flash offered protection in creams having a copper content over 0.75 p.p.m. but under 1.12 p.p.m., while 190° F. flash was only slightly better than 170° F.

6. Oat flour to the extent of two per cent of the fat present offered protection with over one p.p.m. of added copper.

7. Sugar to the extent of 10 and 15 per cent of the cream aided defrosting and reduced "oiling off," but did not improve flavor.

8. Reducing the acidity of frozen cream ice cream mixes to 0.10 per cent practically restored the normal whipping ability.

The paper contains an excellent summary and review of previous published material about the storage of frozen cream and its use as an ingredient of ice cream.

M.J.M.

445. **The Use of Corn Syrup Solids in Ice Cream and Ices.** L. R. GLAZIER AND M. J. MACK, Massachusetts State College, Amherst, Mass. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., 2: 40. Oct., 1940.

In this study the effect of partial replacements of corn syrup solids for sucrose on the flavor, body and textures and other properties of ice cream, has been observed. A slight majority of consumers failed to notice a decrease in sweetness caused by the replacement of 20 and 26 $\frac{2}{3}$ per cent of the sucrose of the mix by corn syrup solids. However, the replacement improved the body and texture in the opinion of about 70 per cent of the people, and the majority preferred the ice cream containing the two sweeteners. Since the most obvious difference between the samples was in body texture, the judges apparently expressed a final preference for the ice cream with the best body and texture.

The results show that sugars have two principal functions in frozen dairy products. One is to contribute sweetness and the other is to improve body and texture. Though sucrose is somewhat sweeter than the corn sweeteners certain combinations of sucrose and corn sweeteners improve body and texture more than sucrose alone. Both of these factors should be considered by the ice cream manufacturer.

Partial replacements of sucrose by corn syrup solids were found to have very little effect on titratable acidity and protein stability, to raise the freezing point slightly, to increase the mix viscosity about 10 per cent, and to affect only slightly the whipping ability of the mixes. The development of sandiness and extreme coarseness, during prolonged storage of ice cream, is delayed by the use of corn syrup solids.

A combination of sucrose and corn syrup solids improved the body and texture of ices and sherbets, as well as ice bars. The change in body and texture produced by the combination of these sugars is more marked in ices and sherbets than in ice cream.

M.J.M.

446. **Detection of Sodium Alginate in Dairy Products.** ANONYMOUS. Food Mfr., 15: 11; 265. 1940.

Sodium alginate may be detected in milk, cream, cream cheese and ice cream by the following method: Boil sample with 6N HCl and wash residue first with 75 per cent alcohol and then with ether until free of soluble matter. Dissolve in dilute NaOH and filter. Precipitate the gum from the filtrate by adding an equal volume of alcohol and wash free of soluble matter with 75 per cent alcohol. Dissolve the purified dried residue in 0.15 cc. of tenth normal NaOH and add 1 cc. of concentrated sulphuric acid saturated with Fe₂(SO₄)₃. In the presence of sodium alginate, a purple color develops after standing.

The weight of residue sufficient to give the color test is 0.1 mg. and

starch, gelatin, Irish moss, agar, gum arabic, formaldehyde, etc., do not interfere with the test. J.C.M.

MILK

447. **Factors Influencing the Flavor of Milk.** P. F. SHARP, Cornell Univ., Ithaca, N. Y. *Dairy World*, 19: 11; 28. April, 1941.

The author presents a rather complete summary of the subject of milk flavors and discusses causes and remedies for the six types of flavor changes: Microbial growth, feed, absorbed, chemical composition, processing and handling and enzymatic and catalytic. F.J.D.

448. **Six Day Delivery from the Plant Angle.** H. D. DRAIN, Peoples Dairy Co., Akron, Ohio. *Dairy World*, 19: 11; 17. April, 1941.

A brief discussion of the plant problems involved in a six day delivery system for fluid milk. The main difficulties involve supplying 40 to 60 per cent more milk for delivery the day before and the day after the day deliveries are omitted; supply and storage of milk; bottles and cases; handling returns; and arrangement of plant working schedules. The author states that in his plant a saving of one and one-half men has been realized and customers have cooperated very satisfactorily. F.J.D.

449. **Frozen Milk.** ANONYMOUS. *Food Mfr.*, 16: 2; 42. 1941.

This is an account of studies made to determine the effect of reconstituted, concentrated, frozen whole milk of (a) degree of concentration, (b) homogenization, (c) length of holding period, (d) temperature of thawing.

Fresh whole milk was pasteurized at 143° F. for 30 minutes, condensed in a small vacuum pan, placed in 2-quart cans, and set in a refrigerator having an average temperature of -10° F. until removed for examination. The homogenized samples were processed at 2,000 lbs. following pasteurization and preceding concentration.

The samples were tested for: flavor, organoleptically; per cent of acidity by titration; creaming ability by allowing to stand in 100 ml. cylinders for 24 hours at 40° F; de-emulsified fat by a method similar to that of Webb and Hall, except that Babcock test bottles were used and the samples were examined after reconstitution; stability in boiling water by adding a few drops of sample to boiling water in a test tube; and appearance of fat separation by visual examination.

The data obtained indicated that milk may be concentrated and held at low temperatures for several weeks without greatly changing its characteristics and quality. A concentration of 3 to 1 appeared to give best results. Tallowy flavor was noted in most of the held samples.

Homogenization gave a final reconstituted product that was smoother in

texture and exhibited less de-emulsified fat. The creaming ability of the milk was completely destroyed and apparently homogenization inhibits development of tallowy flavor.

Curd tension is reduced by each of the processing steps and the two samples of homogenized reconstituted milk tested 0 and 7 grams, while the original milk tested 50 and 47 grams respectively. J.C.M.

450. **Milk Production Trends.** JOHN L. WILSON, Agr. Marketing Service, Washington, D. C. The Assoc. Bull., Internat. Assoc. Milk Dealers, 33rd yr., No. 13; 317-330. Feb., 1941.

With many charts and much factual material the author presents a picture of rather heavy production and good demand during the summer and fall of 1940 with a prospect of low feed costs and increasing numbers of dairy cattle furnishing a heavier supply of milk during the next year or two at least. E.F.G.

451. **A Critical Review of the Phosphatase Test.** L. H. BURGWALD, Ohio State Univ., Columbus, Ohio. The Assoc. Bull. Internat. Assoc. Milk Dealers, 33rd yr., No. 14; 366-389. Feb., 1941.

The test is considered to be accurate for both the holding method and the short time method of pasteurization of milk. It is not quite as accurate for cream and sometimes tests change from negative to positive as the cream ages. Higher temperatures of pasteurization are needed to secure negative tests with ice cream and chocolate milk. Little work has been reported on cheese but these reports indicate it can be applied with a certain degree of accuracy. In the case of butter it appears as if further research is necessary.

This is a very comprehensive review article and has a list of fifty-five references. E.F.G.

452. **A Study of Milk Cooling Systems for the Farm.** F. C. FENTON, Kansas State College, Manhattan, Kans. The Assoc. Bull., Internat. Assoc. Milk Dealers, 33rd yr., No. 13; 341-357. Feb., 1941.

Reports on cooling practices were obtained from 1,144 dairymen in several larger Kansas milk sheds and a detailed study of costs and methods was carried out on the farms of 60 large producers. Of the 76 per cent using some cooling method 40 per cent used well water, 12 per cent used ice and 24 per cent used mechanical coolers of which 28 per cent was wet storage, 48 per cent dry box and 24 per cent walk-in boxes. Of the 60 larger producers surveyed 66 per cent used mechanical coolers of which 44 per cent were wet storage, 30 per cent dry boxes and 26 per cent walk-in units. Well water was used by 21 per cent and ice by 13 per cent. Wet storage units are

typically used by producers who deliver to pasteurizing plants and dry storages by producer distributors.

Milk in 10 gallon cans in dry storage at 41° F. was above 70° F. after 8 hours indicating unsatisfactory cooling. When cooling with 62° F. well water, pumping the water at the rate of 5 gallons per minute, the milk temperature was lowered to 71° F. in 1 hour. Continuous agitation of the milk lowered the temperature to within 2 degrees of the cooling water in 1 hour. With the mechanical wet storage units with 35° F. water agitation of either the milk or water is necessary to reach 50° F. in 13 minutes. If an ice bank is used, a smaller tank will be satisfactory. Complete records were kept for one year on 24 farms. Current consumption per 100 lbs. of milk during four summer months averaged slightly over 2 k.w. hours for both wet and dry storage with the cost of the latter slightly greater. With current at 3 cents per k.w. and ice at 30 cents per 100 lbs. total costs per 100 lbs. for wet storage were 9 cents, dry storage 2.7 cents and ice 40.6 cents. E.F.G.

453. A Study of the Time Temperature Relationships in the Pasteurization of Milk as Regards Creaming, Phosphatase and Bacterial Destruction. F. R. HOLLAND AND A. C. DAHLBERG, N. Y. Agr. Exp. Sta., Geneva, N. Y. The Assoc. Bull., Internat. Assoc. Milk Dealers., 33rd yr., No. 14; 361-365. 1941.

On a small laboratory scale the effect of pasteurization upon creaming ability, phosphatase test and bacterial efficiency were studied at 5° intervals between 140° F. and 175° F.; also including 143° F., 162.5° F., and 172.5° F. It is concluded that the tests to which milk is subjected for the determination of efficiency of pasteurization are too severe for the present standards when used in conjunction with very brief heating periods. It was found that the temperature of 170° F. could be held for 2½ seconds without destruction of cream volume and also at this temperature *Escherichia coli* and phosphatase were inactivated without a holding period at all. It is suggested that it may be possible to obtain the desirable effects of pasteurization at 170° F. for 1 second and at the same time secure a much less cooked flavor than at 143° F. for 30 minutes. E.F.G.

454. Flavors in Milk. JOHN G. DAVIS. Food Mfr., 15: 11; 272-275. 1940.

This is an exhaustive review of milk flavors, their cause and occurrence. Milk flavors may be divided into normal and abnormal. This latter division then breaks down into classifications as physiological, such as feed flavors and excessive cowiness; enzymic, i.e., rancidity; chemical, such as oxidized and cooked flavors; bacterial, which includes souring and fruity flavors; and lastly, mechanical acquired flavors such as chlorine and soap flavors.

Physiological flavors are caused by the feed or pasture; enzymical flavor causes are not very well understood. Chemical change is caused by copper contamination, overheating, etc.; bacterial, by improper care of the milk; and mechanical by contact with foreign substances. J.C.M.

PHYSIOLOGY

455. The Effect of Refeeding and of the Administration of a Pituitary Extract on the Ovaries of Undernourished Guinea Pigs. D. J. STEPHENS AND WILLARD M. ALLEN. *Endocrinology*, 28: 580. 1941.

Undernutrition in female guinea pigs of sufficient degree to cause a loss of 20-30 per cent of body weight in a period of 2 weeks resulted in atrophic and retrogressive changes in ovaries similar in character and degree to those following hypophysectomy. These changes were not affected by the administration of vitamin supplements but normal ovarian structure was restored by refeeding. The ovary of the undernourished guinea pig remained responsive to stimulation by at least one of the hypophyseal gonadotrophic hormones. These results were considered to support the contention that changes occurring in the ovary during inanition may be due, at least in part, to the inability of the anterior pituitary to continue to produce sufficient gonadotrophic hormone to maintain the normal structure and function of the ovary when the food intake is markedly curtailed. R.P.R.

456. Relation of Nutrition to Mammary Growth after Estradiol Administration to Hypophysectomized Rats. L. T. SAMUELS, R. M. REINECKE AND W. E. PETERSEN. *Proc. Soc. Exp. Biol. and Med.*, 46: 379. 1941.

Well nourished by stomach tube feeding, adult virgin hypophysectomized rats did not show mammary development following the administration of 1,000 I.U. of estradiol benzoate every other day for 28 days.

457. Influence of Local Applications of Turpentine on Mammary Gland Growth and Involution. J. P. MIXNER AND C. W. TURNER. *Proc. Soc. Exp. Biol. and Med.*, 46: 437. 1941.

The application of spirits of turpentine for 7 days to the teats and adjoining skin of lactating mice weaned on the 4th day after parturition retarded the rate of involution of the mammary lobule-alveolar systems. Similar applications to spayed and normal females failed to stimulate alveolar growth and pseudo-pregnancy was not produced in the latter group. R.P.R.

458. The Relation between the Fat and Carbohydrate Metabolism of Lactation, as Indicated by the Respiratory Quotient of the Mammary Gland. E. P. REINEKE, W. D. STONECIPHER AND C. W. TURNER, Mo. Agr. Exp. Sta. *Am. J. Physiol.*, 132: 535-541. 1941.

The average respiratory quotient of the active mammary gland in 20 experiments on normal unanesthetized goats was 1.17 ± 0.036 . Local anesthetization (apothesine) in 15 experiments resulted in a mean respiratory quotient of 1.15 ± 0.034 . Use of nembutal as a general anesthetic during the sampling of blood resulted in a mean respiratory quotient of 1.09 ± 0.0115 . Correction for the synthesis of urea in the mammary gland (according to Graham) would increase this figure to approximately 1.18.

The respiratory quotient of the mammary gland of the non-lactating-pregnant goat during the last half of pregnancy was found to be identical with that of the lactating goat, while that of the non-lactating non-pregnant goat had declined to 0.87. Apparently, the increased metabolic requirements of the gland during lactation are met by an increase in the rate of blood flow.

The mammary gland of fasted goats showed a decline in the respiratory quotient to around 0.80, a figure comparable to that reported by other workers for the perfused excised udder.

Because of the parallel between the decline of the respiratory quotient of the mammary gland and the known decrease in the lower fatty acids of milk during fasting, it is suggested that the synthesis of milk from carbohydrate is confined largely to the fatty acids of low molecular weight.

D.E.

459. Androsterone Effect on Pituitary and Mammary Gland. R. P. REECE, New Jersey Agr. Exp. Sta. *Proc. Soc. Exp. Biol. and Med.*, 46: 265. 1941.

Thirty sexually mature rats were ovariectomized and paired on the basis of body weight. One of each pair received subcutaneously daily for 15 days 200 gamma of androsterone. The other one of each pair received no treatment. The hormonal treatment caused no significant change either in pituitary weight or pituitary lactogen content and produced no detectable growth of the mammary glands.

R.P.R.

460. Secretion of Orally Administered Radio-Iron in the Milk of Cows. L. A. ERF, Crocker Radiation Lab., University of California. *Proc. Soc. Exp. Biol. and Med.*, 46: 284. 1941.

Radio-active iron was converted into an iron chloride solution and 115 cc. of this solution administered (by drenching) to 2 cows. One of the cows had previously received 8 gm. of ferrous sulphate twice daily for 12 days. One

of the cows produced 13,350 cc. of milk and secreted an average of .121 per cent of radio-iron per liter of milk, or a total of 1.5 per cent of the amount administered, during the 78-hour period. The second cow (received ferrous sulphate prior to the administration of radio-iron) produced 18,810 cc. and secreted .128 per cent per liter, or a total of 2.5 per cent of the radio-iron administered. It was suggested that "If the average cow producing 4 liters of milk a day can secrete in the milk 0.5 per cent of a 10 gm. oral dose of iron per 24 hours, 12.5 mg. of iron would be present in each liter—an amount more than adequate for a growing child who consumed a liter a day."

R.P.R.

461. **An A. C. Induction Flow Meter for Measurement of Blood Flow in Intact Blood Vessels.** A. KOLIN, Int. Sinai Hospital, New York City. Proc. Soc. Exp. Biol. and Med., 46: 235. 1941.

A modification of the electro-magnetic flow meter was presented. The modification employs an electro-magnet fed by an alternating current. This eliminates disturbances due to polarization and variable galvanic potentials and makes it possible to use an alternating current amplifier.

R.P.R.

MISCELLANEOUS

462. **Cooperative Advertising.** D. T. CARLSON, President, Am. Dairy Assoc., Willmar, Minn. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., 4: 30. Oct., 1940.

The American Dairy Association began its activities in January, 1940. Its purpose is to organize and consolidate on a national basis all state and local agencies for promoting the consumption of dairy products. This is not a duplication of the activities of the National Dairy Council, which are largely educational in nature. The American Dairy Association will carry on commercial advertising of dairy products.

The plan of coverage to be undertaken by the association is advertising through newspapers, radio spots and point-of-sale merchandising in selective markets. The newspaper coverage is now five and one-half millions. Heretofore, advertising has largely emanated from processors through the advertising of specific brands. That type of advertising may succeed in transferring demand from one brand to another but is not effective in increasing *per capita* consumption. By effective cooperative advertising of dairy products the *per capita* consumption of these foods can be increased. M.J.M.

463. **Merchandising as the Dealer Will Accept It.** LOUIS J. WAINER, Penn. Dairies, Inc., Lancaster, Pa. Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs., 4: 37. Oct., 1940.

There are no new and revolutionary principles in merchandising; profitable merchandising therefore consists of putting into practice the things known to be effective. If dealers are to accept one's merchandising plans, these four things must be considered: 1. A carefully planned program; 2. An aggressive program; 3. A simple program which the dealers can easily follow, and 4. The program must have a consistent follow-up plan. A merchandising plan will appeal to dealers when they can see that it will be profitable to them, as well as to the ice cream company.

An effective merchandising plan is the cheapest way to increase gallonage. Increased sales through existing outlets can be realized with less expense than by securing new outlets. M.J.M.

464. **Dusinberre and Oaks Case.** HARRY POLIKOFF. New York Metropolitan Milk Marketing Area. Milk Dealer, 30: 5; 41-44. Feb., 1941.

A discussion is given of the Dusinberre and Oaks case, in which the New York court of appeals sustained the statutory authority of a State control agency to deny a milk dealer's license where no violation of law had been shown, and no intention or attempt to violate. The applicants' lack of experience and "doubtful" prospects were deemed as tending toward destructive competition in a market found to be adequately served and stable without them. C.J.B.

465. **Care and Maintenance of Walls.** L. C. THOMSEN, Dairy Dept., Univ. of Wis. Milk Dealer, 30: 5; 36, 72-74. Feb., 1941.

The importance of light in the dairy plant is stressed. A table is given which shows the percentage of light reflected by colored paints. Other factors discussed are: Dampness and mold growth on walls and ceilings; discoloration of paint; peeling of paint; and tile or glazed brick walls. C.J.B.

466. **Consumer Friendship.** RACHAEL REED, The Borden Co., Chicago, Ill. Milk Dealer, 30: 5; 34, 68-71. Feb., 1941.

A discussion is given to the importance of consumer friendship and how it can be improved through understanding. It is pointed out that there are factors peculiar to the entire dairy industry, at least two of which are complex and require detailed study to be understood. They are: 1. The inter-relation between cost of basic product milk for fluid use and cost of the product for manufacturing purposes. 2. The inter-relation between production costs in different parts of the country. The author believes that the consumer attitude toward price is an educational problem. Other methods of improving consumer friendship are discussed under the head-

ings: "Do not take consumers by surprise," and "Relations with Employees." C.J.B.

467. **Ice at Lower Cost.** ANONYMOUS. *Milk Dealer*, 30: 5; 32, 49. Feb., 1941.

A discussion is given of how some milk dealers are obtaining ice at a lower cost by replacing block ice plants with large-size ice-producing machines. C.J.B.

468. **Accounting in 1941.** FRED PRESTON, The Borden Co., Columbus, Ohio. *Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs.*, 3: 7. Oct., 1940.

The increasing need for the accountant is stressed. Fixed costs are at present rising, hence careful accounting is essential so as to control costs as much as possible. The National preparedness program doubtless will affect plant operations. The added costs of additional taxes, higher labor costs, higher material costs, increased costs of new machinery, trucks, and so on, will make it difficult to cover these added expenses through additional revenues. If this happens, then the only alternative is that of more economical operations. M.J.M.

469. **The Human Side of Advertising.** H. K. DUGDALE, Van Sant, Dugdale and Co., Inc. *Ice Cream Trade J.*, 37: 3; 35. 1941.

An extensive psychological study conducted by one of our leading universities has shown that there are seven basic buying motives or seven primary appeals which if properly used are the most likely to induce people to do what we want them to do. They are: 1. Self-preservation; 2. Profit or love of gain; 3. Appeal to pride or vanity; 4. Appeal to family affection; 5. Civic pride and patriotism; 6. Utility appeal, and 7. Taste. Instead of telling you what a product is and how it works, you are told by advertising what it will do for you, what it has done for other people, why you cannot get along without it, where you can get it, and before you know it you are doing exactly what you were asked to do. W.H.M.

470. **Refrigeration Drives.** E. C. LINDSAY, JR. *Refrig. Engin.*, 41: 3; 173. 1941.

A brief resume of types of mechanical drives used generally in the refrigeration industry to transmit power from the prime mover to the driven machine. They being as follows: 1. Direct connected motors, with or without flexible couplings. 2. Gear drives, including reducers. 3. Chain drives. 4. Flat belt drives. 5. V-Belt drives. L.M.D.

471. **Keep Employees Safe.** EDGAR G. QUESNEL. *Ice Cream Trade J.*, 37: 3; 30. 1941.

Most accidents that have occurred in dairy plants could have been prevented. Dissatisfaction, poor physical condition, lack of interest, lack of consideration for others, acquired or permitted unsafe practices or work habits are the principal reasons which cause accidents. These conditions can be corrected if management can in some way get closer to men and develop a mutual understanding. This can best be done through the medium of interested personal contact.

W.H.M.

472. **Increasing Production Per Kilowatt Consumed.** R. E. MILLER, York Ice Cream Machinery Corp., York, Pa. *Proc. 40th Ann. Conv. Internat. Assoc. Ice Cream Mfrs.*, 2: 74. Oct., 1940.

Some of the features of design and operation tending toward high refrigeration costs are discussed and corrective measures are cited. High refrigeration costs are avoidable, hence a thorough knowledge of their causes and means of prevention are essential.

Corrective measures in one plant may not be suitable in another. Each situation must be carefully studied from the standpoint of its special requirements. Production schedules, power and water rates, and so on, are variables. The only way to determine whether the most is being obtained from refrigeration equipment is by a plant survey conducted by a person competent in solving refrigeration problems.

M.J.M.

473. **The National Institute for Research in Dairying.** T. CROSBIE-WALSH, *Food Mfr.*, 15: 11; 268-271. 1940.

This article describes the National Institute for Research in Dairying and gives some of the work done by it.

The Institute is made up of four main divisions. These are Dairy Husbandry, Chemistry, Bacteriology, and Physiology and Biochemistry. The Experimental Dairy and Library are separate departments though much smaller.

The Institute carries on studies such as the nutritive value of milk and milk products, pasteurization methods and methods of milk analysis. In addition dairy equipment is tested at the Institute.

J.C.M.

474. **War-Time Packaging.** ANONYMOUS. *Food Mfr.*, 15: 12; 292-295. 1940.

This article reviews the field of new packages, new linings and prospective changes in food packaging.

Kraft Cheese Co. introduced a thrift package, a spiral-wound cardboard tube with a slip-over lid. Another Kraft package dispensed with the lid,

covering the cheese with a transparent film over a shallow cardboard tray.

At the start of the war, waxed paper containers were already beginning to be used as "one-trip" receptacles for milk, boiled sweets, etc. The war restricts the use of these containers in the milk and cream industries but greatly promotes their use in other food industries.

A new material called W. 535 has been brought out by Imperial Chemical Industries. This material is used to replace paraffin wax or to increase the usefulness of the paraffin. Due to smaller crystals and voids, the permeability of W. 535 is much less than that of the paraffin wax and when folded a water-permeable crack does not develop. J.C.M.

475. The Thermal Insulating Properties of Silica Aerogel. JOHN F. WHITE. *Refrig. Engin.*, 41: 3; 171. 1941.

A comparatively recent addition to the list of materials being offered to American industry. Silica aerogel is the dried gel obtained by dehydrating silica jelly in such a manner that the solid colloidal structure present in the jelly undergoes no change. It is a light, voluminous solid having a density of 7.0 lb. per cu. ft. and a pore volume of about 94 per cent. Its thermal conductivity is approximately 10 per cent less than that of still air. If the material is opacified its insulating value is enhanced. It may be heated to 1,400° F. without undergoing any change. Very little moisture absorption occurs. Unopacified aerogel will pack to 7.5 lbs. per cu. ft. and the opacified 8.25 lb. per cu. ft. It is particularly suited for cabinet wall insulation, because due to its efficiency, it can be used to decrease wall volume, thus increasing storage capacity within the same over-all dimensions. On the other hand, if used in same thickness as other insulating material a 50 per cent reduction in refrigeration may be made. It is ideal for low temperature insulation. Research is in progress leading to commercial production of a grade that has a density in the order of 3.75 lb. per cu. ft. L.M.D.

476. Moisture, Its Sources, Effects and Control. R. L. HOCKLEY. *Refrig. Engin.*, 46: 3; 179. 1941.

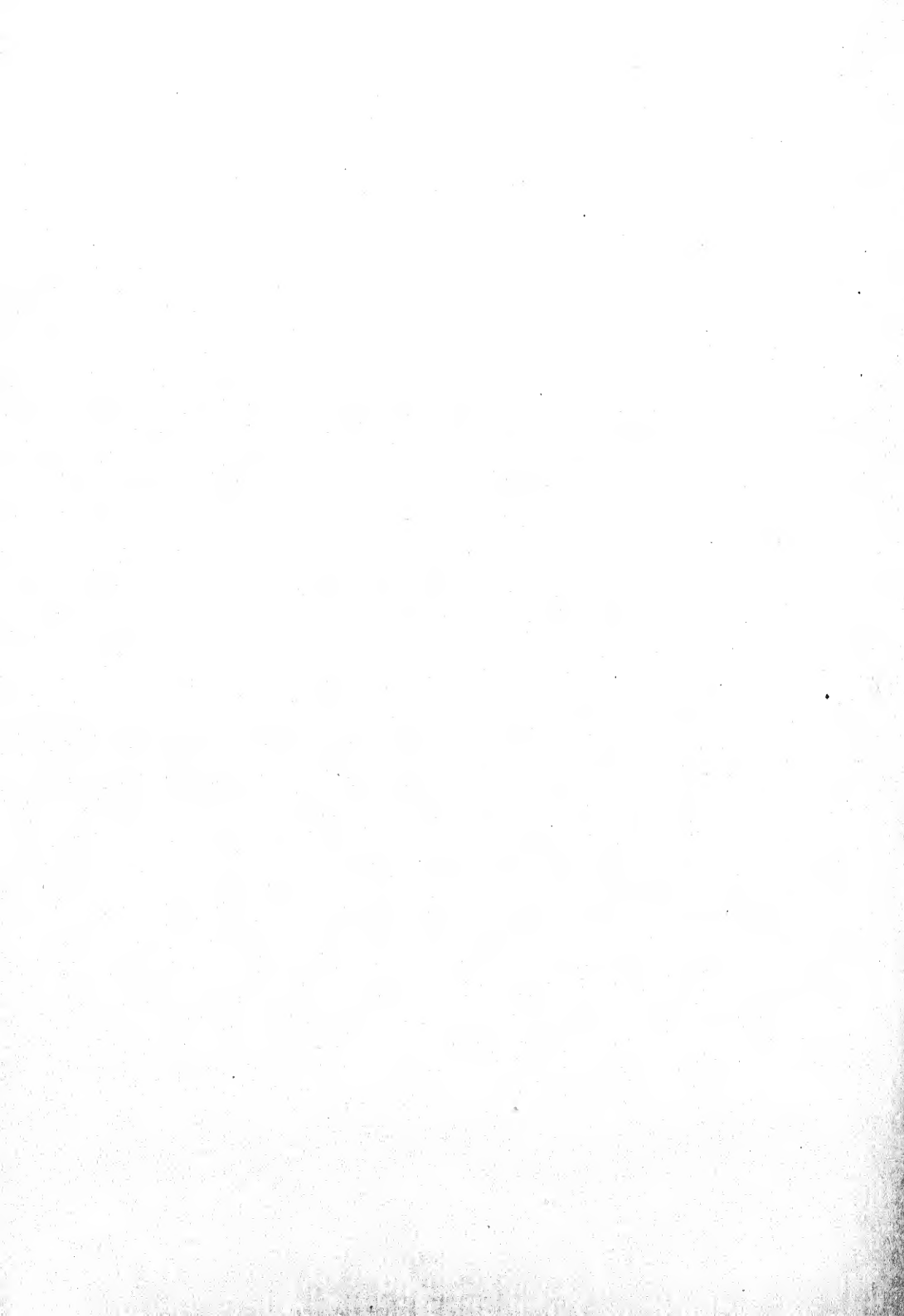
The author lists freeze ups, the formation of sludge, hydrolizing of the refrigerants to form acids, and copper plating as the difficulties resulting from moisture in a refrigeration system. He describes the various methods of installing dehydrators, and the two general types of dehydrating agents, chemical and physical. L.M.D.

477. A Public Relations Program Geared to 1941 Needs. HAROLD W. COMFORT, Borden Co. *Milk Dealer*, 30: 5; 112-119. Feb., 1941.

The reasons for some of the opinions regarding the milk business which are now entertained by the public are given and means of correcting these

opinions are discussed. The author concludes his discussion as follows: "In developing better public relations, we should always keep the fundamentals in mind lest we lose the substance by grasping at shadows. Let us continue developing sound operating policies to meet changing conditions; let us put our house in order—if corrective measures are needed. And then let us demonstrate conclusively to all the people who make up our various publics that this industry operates in complete harmony with public interest."

C.J.B.



JOURNAL OF DAIRY SCIENCE

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New York Association of Dairy and Milk Inspectors	United States Department of Agriculture

ABSTRACTS OF LITERATURE

ADVANCE ABSTRACTS OF REPORTS ACCEPTED FOR PUBLICATION

478. The Relationship of pH to Some Curd Characteristics of Modified Milks. ARNOLD B. STORRS, American Seal-Kap Corporation, Long Island City, N. Y.

A study has been made by means of the Chambers-Wolman "artificial stomach" test of the effect of variations in pH upon the curd surface area, the bulkiness and the completeness of curd formation of some commercially modified milks. The curd surface area of any milk appears to be lowest at the highest pH level at which complete coagulation will first appear, while at any pH below that required for complete coagulation the curd surface area increases as the pH is lowered. The effect of pH upon the bulkiness or completeness of curd formation is variable in milks modified by different processes and the method of modification seems to be the most important factor in determining the relationship. Because of the varying response of different types of milk there does not seem to be any single pH level which, in view of our present knowledge, would be suitable for comparative *in vitro* tests on all milks.

479. Observations on Delayed Salting of Brick Cheese. W. L. LANGHUS AND W. V. PRICE, University of Wisconsin, Madison.

Manufacturers have attempted to hasten the ripening of Brick cheese by delaying the salting operation for several days. Existing information indicates that this treatment may affect the rate of curing as well as flavor, body, texture, and color finally attained. The idea was investigated by salting, at intervals of 1, 5 and 9 days after manufacture, cheese made from either raw or pasteurized milk.

When the salting of cheese is delayed there is an improvement in cheese body apparent at two weeks of age, but this advantage eventually disappears and the general quality of the cured cheese is not as good as that of the cheese salted in the normal manner. The delayed salting does not materially affect the amount of salt incorporated in the cheese nor the acidity of the cheese during ripening but it does increase the loss of moisture during the first two weeks of curing. The differences in body apparent during this interval are attributed to changes in the protein rather than to differences in cheese composition.

Normal addition of salt soon after making establishes conditions favorable to the development of desirable flavor and body. Delayed salting, as practiced in these experiments, has no lasting benefits to commend it to the Brick cheese industry.

480. **A Double Change-over Design for Dairy Cattle Feeding Experiments.** W. G. COCHRAN, K. M. AUTREY, AND C. Y. CANNON, Iowa Agricultural Experiment Station, Ames, Iowa.

During the winter of 1939-40, a short-time feeding trial, of the double switch-over type, was carried out on eighteen Holstein cows, with three planes of feeding: roughage, limited grain and full grain. Every cow received each ration in turn for a period of six weeks, while in any period one-third of the cows were receiving a given ration, so that differences between the milk yields of different cows and differences between the average yields for the three periods did not contribute to the experimental errors. With this design accurate comparisons between the effects of the three rations were obtained.

The design also made it possible to estimate the sizes of the carry-over effects of the rations from one period into the succeeding period, and to adjust for these where necessary. By failure to adjust for carry-over effects on the yields of fat-corrected milk, the differences between the rations would have been underestimated by about 11 per cent.

The computations required to estimate the experimental errors are illustrated by using the results for total nutrient consumption, where there appeared to be no carry-over effects, and for fat-corrected milk, where an adjustment for carry-over effects was necessary.

A corresponding design for comparing four planes of feeding is briefly discussed.

481. **Estimation of Initial Live Weight at Each Lactation of Dairy Cows.** W. L. GAINES, Illinois Agricultural Experiment Station; H. P. DAVIS AND R. F. MORGAN, Nebraska Agricultural Experiment Station.

From measurements of chest girth and live weight at the Nebraska Station an equation has been derived for estimating initial live weight, that is, within the first 31 days after calving. The equation is $W = .342(G + g)^{1.85}$ in which W is weight in pounds, G is chest girth in inches, and g is an age-breed girth modifier, ranging from zero for Jersey cows under 3 years of age up to 9 for Holstein cows 5 years or more of age. A table, based on the equation, is printed on the case of the stock 96-inch steel tape rule used to measure the girth. As compared with the present scale the scale of the New York girth-weight tape grossly overestimates the weight at large girths and grossly underestimates it at small girths.

482. **The Effect of Processing on the Nitrogen Distribution in Milk.** S. G. MENEFEE, O. R. OVERMAN, AND P. H. TRACY, Department of Dairy Husbandry, University of Illinois, Urbana, Illinois.

Semimicro methods were used to make a preliminary investigation of the

nitrogen distribution in processed milk products and determine the routine applicability of semimicro methods for separating and analyzing the N fractions of milk.

The homogenization of milk at normal and abnormal pressures produced no significant changes in the N distribution.

The coagulation of albumin and globulin was the most significant change that occurred in the N distribution of evaporated milk. Evidence is presented to indicate that some hydrolysis of the proteins takes place in this product as a result of the processing.

Condensing skim milk produced only minor changes in the N distribution and the addition of Steapsin, Trypsin, and Enzylac to milk produced definite hydrolysis of the milk proteins.

The semimicro methods used for the analytical determinations were found to be efficient, well adapted to routine analysis and results compared very favorably with those obtained by the official methods.

483. *Pseudomonas putrefaciens* in Dairy Plant Equipment. H. F. LONG and B. W. HAMMER, Iowa State College, Ames.

Pseudomonas putrefaciens, which is a common cause of the putrid defect in butter, was isolated from churns and from the insulation of a leaky vat. At certain points the organism was present in considerable numbers. In addition to *Ps. putrefaciens*, micrococci, spore forming bacteria, gram negative rods and often yeasts and molds were present.

484. Oxidized Flavor in Milk IX. The Effect of the Quality of Hay and Early Stage of Lactation on the Carotene Content of Butter Fat and the Ascorbic Acid Content of the Milk and Their Relationship to the Development of Metal-Induced Oxidized Flavor. W. CARSON BROWN, A. H. VANLANDINGHAM, AND CHAS. E. WEAKLEY, JR., West Virginia Agricultural Experiment Station, Morgantown.

A study of oxidized flavor in milk produced on different qualities of alfalfa hay revealed very little relationship between the carotene content of the butter fat and the intensity of the oxidized flavor developed. Likewise, no relationship was found between the ascorbic acid content of the milk and the intensity of the oxidized flavor developed. A study of the early stages of lactation revealed that the ascorbic acid content of the milk at the beginning of lactation was relatively low and increased for approximately seven to eight weeks after which it remained fairly constant. The carotene in the butter fat decreased during the first few weeks of lactation, while the intensity of oxidized flavor remained fairly constant. As the result of 580 observations (each observation consisted of three determinations per week) on the ascorbic acid content of the milk, and 555 observations (each observation

represents a composite of three samples per week) on the carotene content of the butter fat it was concluded that:

1. The feeding of high quality alfalfa hay together with alfalfa leaf meal increased, somewhat, the carotene content of the butter fat and greatly reduced or eliminated the tendency for metal-induced oxidized flavor to develop.

2. The feeding of brown, leafy alfalfa hay resulted in a decreased carotene content in the milk but did not increase the intensity of the oxidized flavor.

3. The carotene content of the milk fat at the beginning of lactation appears to be high and decreases until it reaches a normal level a few weeks after parturition.

4. The ascorbic acid content of milk at the start of lactation is usually low and increases gradually until it reaches a maximum level at about seven or eight weeks following parturition.

5. From the results obtained it appears that ascorbic acid in the milk plays a minor role in the susceptibility of the milk to metal-induced oxidized flavor.

6. The results of this study indicate that the amount of carotene in the butter fat may not be the substance responsible for the reduction in susceptibility of milk to oxidized flavor. It appears that some substance or substances associated with carotene probably has a greater effect than the carotene itself.

485. A New Diluent for Bovine Semen. C. E. KNOOP, Dairy Department, Ohio Agricultural Experiment Station, Wooster, Ohio.

A diluent for bovine semen which contains gelatin (Knox), egg yolk, buffer salts, and water has been found, under improved conditions, to maintain the motility of the spermatozoa as follows: After two to four days in storage the motility averaged 73 per cent (range 57 to 83 per cent); motility after 12.5 days was 50 per cent; and motility after 17.5 days was 25 per cent. Some cell life was observed for an average of 26.5 days (range 16 to 35 days).

The motility of a comparable group of semen samples diluted with a diluent containing egg yolk, buffer salts, and water averaged after two to four days in storage 59 per cent (range 33 to 80 per cent), after 8.3 days, 50 per cent, and after 14.5 days, 25 per cent. Some sperm life remained for an average of 26 days (range 14 to 38 days).

BACTERIOLOGY

486. The Maintenance of Pure Cultures of Lactic Acid Bacteria. J. G. DAVIS, Dairy Indus., 6: 8. 1941.

A detailed consideration of pure cultures of lactic acid bacteria including isolation, media, routine maintenance, contamination, detecting contami-

nation, purification and recovery of apparently dead cultures. Methods and techniques are described for isolation and maintenance of such cultures.

D.V.J.

487. **Bacteriology of Dairy Water Supplies.** A. L. PROVAN, Dairy Indus., 6: 65. 1941.

Farm and dairy water supplies should be of a standard equal to that demanded for domestic usage and in addition should not contain organisms which are liable to cause rapid deterioration in dairy products. The beneficial effect of proper protection of farm water supplies is demonstrated by a bacteriological study in which farm waters were analyzed before and after protection.

Although chlorination is the simplest method of control, it does require considerable technical control and is frequently more costly than other control measures.

D.V.J.

488. **A Presumptive Test for the Oral Contamination of Drinking Utensils.** LEO A. DICK AND G. J. HUCKER, Dept. of Bacteriology, N. Y. State Agr. Exp. Sta., Geneva, N. Y. Jour. Milk Tech., 3: 307-313. 1940.

"Total bacterial count" of eating utensils gives inadequate information as to the sanitary condition of eating and drinking utensils. More attention must be given to qualitative methods for determining the sanitary condition of such utensils.

A procedure is given for the detection of oral contamination of drinking glasses. Conclusions arrived at in this study were as follows:

The presence of *Streptococcus salivarius* on the rims of drinking glasses indicated that the glasses had not been properly washed after use. This organism was recovered from the closed lips of each of 100 persons tested; and without exception, in 100 controlled cases the organism was deposited on the rims of glasses during use.

When glasses are not cleaned or sterilized after use, this organism was found to survive thereon for at least 48 hours.

A cold water rinse following use will not remove this organism, neither will a double rinse of water at 120° F. be adequate.

A soap water wash (pH 8.6) at 120° F. followed by a rinse in clear water at 165° F. for five minutes will do the job providing the contamination is not too great.

The presence of this organism on the rims of drinking glasses is an indication of previous oral contamination.

L.H.B.

489. The Effect of Variations in the Fat Percentage and in the Reaction (pH) of Milk Media on the Heat Resistance of Certain Milk Bacteria. AGNES A. NICHOLS, The Hannah Dairy Res. Inst., Kirkhill, Ayr., Scotland. Jour. Dairy Res., 11: 274-291. 1940.

A comparison was made of four different types of containers, namely, (a) glass capillary tubes, (b) glass ampoules or bulbs, (c) glass Pasteur pipettes and (d) corked test tubes as a container for the inoculated substrate for heat resistance tests. The cork test tube was considered most satisfactory. Two strains of *B. subtilis* which had produced bitterness and thinning in canned cream were used in a study of the effect of the reaction on their destruction by heat. The results expressed as percentage survival were inconclusive over the range studied, pH 5.95 to 7.0 in one case and pH 6.1 to 7.25 in another. No consistent differences in survival percentages were noted using substrates of skim milk, whole milk, 10 per cent butterfat cream and 23 per cent butterfat cream. S.T.C.

490. The Microbiology of Silage Made by the Addition of Mineral Acids to Crops Rich in Protein. II. The Microflora. A. CUNNINGHAM AND A. M. SMITH. College of Agr., Edinburgh, Scotland. Jour. Dairy Res., 11: 243-265. 1940.

The microflora of A.I.V. silage was found to consist mainly of lactic acid bacteria—lactobacilli, streptococci, micrococci and sarcinae. A detailed study was made of some 70 strains. The characteristics which the authors considered to be most valuable for the differentiation of the organisms were ability to produce carbon dioxide, percentage of lactic acid formed and lactic, acetic acid ratio.

Among the lactobacilli, both homo- and heterofermentative types were represented. The former included strains of *Lactobacillus plantarum* (Orla-Jensen) Bergey *et al.* and in the heterofermentative group *L. brevis* (Orla-Jensen) Bergey *et al.* was found. Cultures of a motile homofermentative lactobacillus were isolated. This was considered to be a new type.

The homofermentative streptococci were found to belong to the *Streptococcus lactis* group; and the heterofermentative forms were identified with *Leuconostoc mesenteroides* (Cienkowski) van Tieghem.

Streptococci, micrococci and motile lactobacilli were found mainly in fodder recently ensiled, while the majority of the lactobacilli and sarcinae were associated with the older samples. S.T.C.

BREEDING

491. Causes of Variation in Milk and Butterfat Yield of Dairy Cows. IVAR JOHANSSON AND ARTHUR HANSSON. (In English) Jour. Roy. Swedish Acad. Agr., Jahrg., 79, No. 6½. 127 pp. 1940.

Some 7,000 lactation records from 3,000 cows in 13 herds of Swedish Red

and White cattle were studied to measure the importance of various non-genetic factors (such as age, dry period, length of calving interval, etc.) and of genetic differences in causing milk and fat production to be high or low. Correction factors for the more important recorded non-genetic factors were devised. Intra-herd repeatability of differences in single lactation records was +.36 among the corrected records of some 300 cows which each had at least five records. The Intra-herd correlation between fat percentage and milk yield was -.17 but averaged practically zero within groups of records by the same cow. The genetic portion of the variance in single records was estimated to be: 70 to 80 per cent for fat percentages, 30 to 40 per cent for total milk or fat yield, 15 to 30 per cent for persistency, and zero to 5 per cent for length of calving interval. J.L.L.

BUTTER

492. Butterschmalz, eine nationale fettreserve. (Butter Oil, a National Fat Reserve.) H. BALLHOFER. Deut. Molkerei. Ztg., 44, 1443-1444. 1939.

This article explains mass production of butter oil. Originally this procedure was a small scale operation. Today because of a national emergency it is urged that the operation be adjusted to a daily capacity of 400,000 pounds at large plants. This huge capacity is suggested so as to quickly convert butter stocks into oil as a vitamin saving venture. J.C.M.

493. Versuche über Herstellung und Haltbarkeit von Butterschmalz. (Experiments Dealing with the Manufacture and Keeping Quality of Butter Oil). M. SCHULZ AND W. STORCK. Deut. Molkerei Ztg., 9: 29 and continued in 10: 143-145, and in 11: 166-167. 1940.

Butter oil was obtained by mixing butter with water and centrifuging it. Some was centrifuged at 57° C. (134.6° F.) and then heated to 90° C. (194° F.). This kept well in cold storage for 2 years.

It was discovered that 15° C. (59° F.) was a better storage temperature than 5° C. (41.9° F.) for butter oil. At the lower temperature of storage texture defects were observed.

It was also recommended to add .5 per cent of oat flour to the butter oil to be stored. J.C.M.

494. Die Verbutterung von Süsзраhm und die Verwendung der süszen Buttermilch in der Käseerei. (The Churning of Sweet Cream and the Use of Sweet Buttermilk in the Cheese Factory.) W. RIEDEL. Deut. Molkerei Ztg., 2: 11 and continued in 3: 18. 1940.

The author reviews briefly the history of sour cream butter in Southern Germany. He emphasizes the fact that this butter is typically oily, metallic and sometimes fishy in flavor.

Experiments were conducted churning at pH 5 to avoid the flavor defects and achieve the sour cream butter aroma. This was achieved by adding 2 per cent of culture at churning time, allowing no time for acid development. The cream best suited for this contained 35 to 40 per cent of fat. Churning was at 4° C. (39.2° F.); and the buttermilk was at 9° C. when drawn from the churn.

The above procedure gave a sweet cream buttermilk high in fat. This was very suitable for mixing with milk to make a 20 per cent fat Edam type cheese. It required 20 per cent of buttermilk and a setting temperature 2° higher to achieve good results with this procedure. A high fat content Edam type cheese was made with 20 per cent of buttermilk added to normal milk fortified with cream.

The cheeses made from sweet buttermilk and whole milk were equal in quality to the orthodox skim milk and milk mixture made cheeses.

Romadur and semi-soft surface ripened cheeses were also made successfully with the sweet cream buttermilk—milk mixtures, which by itself had little value for cheese purposes. J.C.M.

495. **Keeping Quality of Butter.** O. F. HUNZIKER, La Grange, Ill. Natl. Butter and Cheese Jour. 32, No. 5: 12. May, 1941.

Keeping quality is attained best by using high quality cream; by eliminating contamination through adequate plant and equipment sanitation and proper pasteurization; by thorough working of butter; and by protecting cream and butter from contact with iron and copper surfaces. Salted butter made from high acid cream is more susceptible to chemical deterioration than unsalted butter; the latter is more easily damaged by bacteriological changes. Ripening cream to .35 to .45 per cent acidity improves the keeping quality of unsalted butter. This type of butter keeps well in -15° F. storage but at temperatures above freezing deteriorates more rapidly with increasing temperatures. W.V.P.

CHEESE

496. **Pultost, (Ramost).** L. FUNDER. Meieriposten, 20: 360-361. 1940.

This article describes Pultost (Ramost) a Norwegian cheese little known and made from skimmilk and milk.

The skimmilk is mixed with 10 per cent of whole milk and cultured with 2 per cent of starter for several days. The acidity usually reaches .7. At this point the mixture is heated slowly to 55-60° C. (131-140° F.) and held for several hours. It is then drained. Salt is added at a 4 per cent rate and caraway is added in small dosages. The cheese is consumed when fresh or after aging. It is a rare cheese and only consumed by occupants of thinly settled regions. J.C.M.

497. Studies on the Chemistry of Cheddar Cheesemaking. VII. The Measurement of the Acidity of Cheese and the Relation of Acidity to Grading Score. R. M. DOLBY, F. H. McDOWALL, AND W. RIDDET, Dairy Res. Inst. (N. Z.), Palmerston North, New Zealand. Jour. Dairy Res., 11: 305-310. 1940.

Data were secured on 420 cheese comprising a complete season's make at the institute experimental factory. The pH of cheese at 14 days old was considered to be the most useful means of measuring the extent of acid development in the cheese. Values on younger cheese were less stable because the acidity was still increasing. A fair agreement was found between extent of acid development as indicated by pH of the 14-day old cheese and observations of the graders on the mature cheese. Cheese with a pH value of 4.90 at 14 days received the highest average score at maturity. S.T.C.

498. What About Foreign Type Cheese? H. G. LINQUIST, Mass. State College, Amherst, Mass. Natl. Butter and Cheese Jour., 32, No. 5: 16. May, 1941.

Cheese consumption has increased since 1934 and recently imports have been reduced. Swiss cheese of good quality must and can be made in the United States. Roquefort type made from cow's milk is being made and cured successfully in abandoned coal mines and sandstone caves. Domestic Edam and Gouda and Italian types of cheese are being readily accepted. American industry can make the foreign types if care is taken to study markets and practice the best methods of manufacturing and curing.

W.V.P.

499. Practical Suggestions on the Use of Rennet. M. W. HALES, Chris Hansen's Lab., Milwaukee, Wis. Natl. Butter and Cheese Jour., 32, No. 4: 26. 1941.

Many factors affect formation of curd by rennet; among them are temperature of coagulation; salt content of the milk, particularly calcium; use of milk from diseased udders; per cent of casein and fat in milk; milk acidity; and agitation of milk during coagulation. The rennet test, when properly used, is a good measure of change in milk acidity and can be used to compare strength of two lots of rennet when identical milk and coagulation temperatures are used for the tests. Good commercial rennet extract kept in a closed vessel decreases in strength 3 per cent per month in a warm room; in a cool cellar it loses 1 per cent per month; and in cold storage the loss is even less. If temperatures are too high the rennet may spoil. The container used to measure cheese color should be rinsed out before using it to measure rennet because alkaline cheese color weakens rennet action.

W.V.P.

500. The Influence of "Mastitis" upon the Yield and Quality of Cheddar Cheese. C. K. JOHNS, T. J. HICKS, AND C. A. GIBSON, Dept. of Agr., Ottawa, Canada. Jour. Dairy Res., 11: 298-304. 1940.

Milk from the animals in the Central Experimental Farm herd was grouped as follows:

A. Normal—negative daily strip-cup record; no evidence of bacterial infection; catalase low.

B. Abnormal—strip-cup record negative or rarely mildly positive; frequent high values for catalase and pH; *Str. agalactiae* absent from repeated samplings.

C. *Agalactiae*—*Str. agalactiae* previously isolated from milk on at least two occasions; strip-cup rarely positive; latent infection with no pathogens detected during experimental period.

The yield of Cheddar cheese from the milk classified as normal was greater than that from the other groups. Evidence was secured indicating that the lowered yield resulted from the lower casein and solids-not-fat contents of the "mastitis" milks.

With a single exception the cheese made from these "mastitis" milks were not inferior in quality to those made from the normal milks. S.T.C.

CHEMISTRY

501. Untersuchungen über die Genauigkeit der Milchfettbestimmung nach dem Gerber-Verfahren und ihre Grenzen (Studies Dealing with the Accuracy Obtained in Fat Analyses by the Gerber Method). G. ROEDER, Milchw. Forsch., 20: 200-256. 1940.

The author studied the accuracy of the glassware used in the Gerber Test. He also studied the practical use of this test which is being universally used for fat analyses.

It was possible to obtain results that checked closer than 0.1 per cent. However, 0.1 is set as the acceptable variation in duplicate samples. Occasionally duplicates varied as much as 0.25 per cent.

This study is a duplicate of like studies conducted in the United States with the Babcock Method. The percentage of accuracy as measured is quite comparable for both tests.

J.C.M.

502. A Spectroscopic Method for the Quantitative Estimation of Vitamin D. NICHOLAS A. MILAS, ROBERT HEGGIE, AND J. ALBERT RAYNOLDS, Mass. Inst. Tech., Cambridge, Mass. Jour. Indus. and Engin. Chem., Analyt. Ed., 13, No. 4: 227-231. 1941.

Two new and independent procedures for the estimation of vitamin D in fish liver oils are described. One is based on the spectrophotometric esti-

mation of the extinction coefficients at 500 to 520 m μ of the color produced when the vitamin D of the fish oil in chloroform is added to a solution of antimony trichloride in chloroform. Corrections are made for the presence of sterols and vitamin A, both of which interfere with the vitamin D adsorption band. The second method is based on a chemical treatment of the non-saponifiable fraction of fish liver oils with maleic anhydride to destroy vitamin A, carotenoids, and possibly 7-dehydrocholesterol. The vitamin D in the treated non-saponifiable portions is estimated spectrophotometrically. The results are considered to be in fair agreement with the biological. B.H.W.

503. Chemical Estimation of Nicotinic Acid and Vitamin B₆. HARRY A. WAISMAN AND C. A. ELVEHJEM, Univ. of Wis., Madison, Wis. Jour. Indus. and Engin. Chem., Analyt. Ed., 13, No. 4: 221-225. 1941.

A review of the factors involved in the chemical determination of nicotinic acid and of vitamin B₆ is presented. Chemical methods for the estimation of nicotinic acid depend upon a breakdown of the pyridine ring structure generally with cyanogen bromide and conjugation of the carbon chain with an aromatic amine, generally aniline. The color formed from nicotinic acid, cyanogen bromide and an amine is highly specific. The conditions of the reaction are discussed and it is concluded that the method can be applied with a fair amount of success to the determination of nicotinic acid in animal tissues, urine and blood but it is not so well adapted for use in the analysis of plant materials. The chemistry of three methods for the determination pyridoxine (vitamin B₆) is discussed and equations for the reactions are presented. B.H.W.

504. Chemical Methods for Determination of Vitamin C. C. G. KING, Univ. of Pittsburgh, Pittsburgh, Pa. Jour. Indus. and Engin. Chem., Analyt. Ed., 13, No. 4: 225-227. 1941.

Most of the methods for the estimation of vitamin C are based on the reversible oxidation of ascorbic acid to dehydroascorbic acid. The reaction of ascorbic acid with 2, 6-dichlorophenolindophenol can be used in direct titrations or with the photoelectric colorimeter with satisfactory results. Other methods are available for use under special circumstances. Methods for the measurement of dehydroascorbic acid are subject to the interfering reaction of many aldehydes, ketones, and quinones. Three methods for detecting and avoiding such interference are pointed out. B.H.W.

505. Chemical Methods for Determination of Vitamin B. DOUGLAS L. HENNESSY, Fordham Univ., New York, N. Y. Jour. Indus. and Engin. Chem., Analyt. Ed., 13, No. 4: 216-218. 1941.

In a review of methods for the determination of Vitamin B₁ it is con-

cluded that accurate and rapid chemical methods are available for the determination of this vitamin. B.H.W.

506. Recent Developments in Methods for Determining Carotene. WALTER J. PETERSON, Kansas Agr. Exp. Sta., Manhattan, Kansas. Jour. Indus. and Engin. Chem., Analyt. Ed., 13, No. 4: 212-216. 1941.

Improvements in methods for the extraction and quantitative determination of β carotene in dry and fresh plant tissue are described. Solvent and adsorption methods for the separation of β carotene from accompanying petroleum-soluble carotenoids are discussed. Refinements in the technique of column preparation in chromatographic adsorption are presented.

B.H.W.

507. Photoelectric Photometer for Vitamin A Estimation. ALLAN E. PARKER AND BERNARD L. OSER, Electrical Testing Labs., New York, N. Y., and Food Res. Labs., Long Island City, N. Y. Jour. Indus. and Engin. Chem., Analyt. Ed., 13, No. 4: 260-262. 1941.

An instrument for determining the spectral absorption of light in the ultra-violet and by which a rapid and accurate estimation of vitamin A can be made is described. Static conditions are maintained in an electrometer tube by holding the grid potential at a constant value. This is done by the use of a slide-wire potentiometer. The readings on the calibrated slide wire give either the transmission of the cell and material or the extinction coefficient. The instrument is economically constructed, readily portable and by the use of a wide range of light sources and filters it may be used for making transmission measurements in other ranges of the ultra-violet. B.H.W.

508. Determination of Vitamin B₂ (Riboflavin). Comparison of Bioassay, Microbiological, and Fluorometric Methods. A. D. EMMETT, O. D. BIRD, R. A. BROWN, GAIL PEACOCK, AND J. M. VANDENBELT, Res. Lab., Parke, Davis & Co., Detroit, Mich. Jour. Indus. and Engin. Chem., Analyt. Ed., 13, No. 4: 219-221. 1941.

The results of a comparative study of four methods for determining vitamin B₂ are presented. The methods were applied concurrently to several samples varying in type, composition, and potency. The four methods, biological rat growth, visual fluorescence, photoelectric fluorescence and microbiological by both culture turbidity and acidimetry gave similar results. The greatest differences were with the low-potency samples. The microbiological method showed excellent specificity and reproducibility and the results of measurements of culture turbidity at 24 hours and of acidimetry at 72 hours incubation were almost identical. B.H.W.

509. **Physical and Chemical Determination of Vitamin A.** J. B. WILKIE, U. S. Food and Drug Admin., Wash., D. C. *Jour. Indus. and Engin. Chem., Analyt. Ed.*, 13, No. 4: 209-211. 1941.

A review of the current status of the physical and chemical methods for the determination of vitamin A is presented. The use of the antimony trichloride reaction and of ultraviolet absorption for estimation of vitamin A is discussed and factors affecting the stability of the vitamin are considered.

B.H.W.

510. **Measuring Oxidation of a Vegetable Oil.** GEORGE L. CLARK AND FRANK M. RUGG, Noyes Chem. Lab., Univ. of Ill., Urbana, Ill. *Jour. Indus. and Engin. Chem., Analyt. Ed.*, 13, No. 4: 243-244. 1941.

Measurement of the spreading pressure of drops of soybean oil placed on a monomolecular film on a hydrophilic balance has been used to evaluate the oxidation of the oil. The results are compared with the peroxide number and are considered to be more accurate than the peroxide number in the evaluation of oxidation. The method is probably applicable to other liquids which contain hydrophilic groups such as lubricating oil addition agents.

B.H.W.

511. **On the Use of Various Sera for the Determination of Soluble Calcium and Phosphorus of Milk.** G. T. PYNE, Dairy Chem. Dept., University College, Cork, Ireland. *Jour. Dairy Res.*, 11: 292-297. 1940.

The author reaches the following conclusions with respect to the value of various milk sera for the determination of the soluble phosphate and calcium of milk. Dialysis at 0-5° C. (32-41° F.) was used as the standard method:

1. Rennet whey was the most satisfactory; with fresh milk it yields fairly accurate values for calcium, slightly low (average 5 per cent) for phosphate. With increasing acidity of the milk both sets of values tend to rise somewhat relatively to those of the dialysate, phosphate approaching the true value and calcium rising slightly above it.

2. Mercuric chloride borax serum gives high results for the soluble phosphate of fresh milk. With increasing acidity of the milk, the results fall steadily.

3. Papain whey gives somewhat low results for both the phosphate and calcium of raw and pasteurized milk, with the same tendency as rennet whey to yield higher and improved results as the acidity of the milk increases. With boiled milk it gives abnormally high results for both constituents.

The additional phosphate and calcium appear to arise from the solution of colloidal calcium phosphate. S.T.C.

DISEASE

512. **Drei Jahre Eutergesundheitskontrolle in Schleswig-Holstein.**
(Three Years of Udder Infection Control in Schleswig-Holstein.)
A. HEINKE. *Tierzüchter*, 29: 281-283. 1940.

A total of 72,000 animals are being studied. Samples of 100 cc. size are taken from each quarter at the start and close of the milking. The control is very rigid since the war started. Vigilance is essential with the war order changes involving the use of more milking machines and personal changes. Each animal is tested twice annually.

The program also calls for points of sanitation beyond the cows.

The udder samples are inspected for evidence of mastitis. Tuberculosis and abortion are followed closely. Diseases of the cow typical of the area are also followed.

The essence of the entire program is to completely eliminate by slaughter diseased animals. J.C.M.

513. **Elimination of Streptococci from Superficial Wounds by Sulphanilamide Powder.** LEONARD COLEBROOK AND A. E. FRANCIS, Royal Army Medical Corps. *Lancet*, 240: 271-273. 1940.

The moist wounds after a saline bath were sprinkled with sulphanilamide powder and covered with a moist dressing that was kept moist with a larger sheet of jaconet or oiled silk and a firm bandage. In 21 cases with Lancefield's Group A streptococci, in one case with Group C streptococci, and in 2 cases with Group G streptococci, local application with sulphanilamide resulted in permanent disappearance of the organisms. Four of these cases received simultaneous oral administration of sulphanilamide. Four cases with Group D streptococci resisted the local treatment. Neither sulphanilamide nor sulphathiazole had consistent effects on staphylococci, *B. proteus*, or *Pseudomonas pyocyanea*. J.F.C.

514. **Sulphathiazole in Treatment of Staphylococcal Infections.** GEORGE MELTON, Lewisham Hospital, London County Council. *Lancet*, 240: 274-278. 1940.

In a series of 50 cases of staphylococcal infection sulphathiazole treatment appeared to be beneficial in reducing toxemia and in preventing extension of the infections. J.F.C.

515. **The Percutaneous Tuberculin Test.** F. DUDLEY HART. *Lancet*, 240: 414-415. 1941.

Percutaneous tests were performed on nearly 1400 subjects of different

age-groups. Seven hundred of the subjects in the age-group of 15 and under were tested in addition by the Mantoux intradermal test. Of the remaining subjects many of the non-reactors were tested by the Mantoux test for confirmation. The patch test gave a high degree of agreement with the intradermal test with subjects under 16 years of age. With the older age-groups, the patch test became less reliable. J.F.C.

516. *Bacillus lactis aerogens* Infection in the Newborn. JEAN M. CASS. *Lancet*, 240: 346-347. 1941.

An outbreak of acute infection occurring in the nursery of a maternity hospital was attributed to *Bacillus lactis aerogenes*. Of 22 infants, 5 were affected, 2 of whom died. *B. lactis aerogenes* was found in pure culture in the blood of the 2 fatal cases at autopsy and was the predominating organism in the stools of all cases. Three infants, who were acutely ill had never received breast milk, and the other 2, who suffered only mild attacks, had received only a little breast milk. All other infants in the nursery at the time were breast-fed. Although *B. lactis aerogenes* could be isolated from the stools of some of the healthy infants, it was never found as the predominating organism.

The organism was V.P. negative, M.R. negative, and citrate positive. It fermented lactose, glucose, saccharose, mannite, salicin, glycerin, cellobiose, and inosite, and failed to ferment dulcete, inulin, indole, and gelatin.

J.F.C.

517. Subclinical *Staphylococcus* Mastitis in Herds Free From *Streptococcus* Mastitis, and Its Effect upon Milk Composition. P. M. F. SHATTOCK AND E. C. V. MATTICK, Natl. Inst. for Res. in Dairying, Univ. of Reading, England. *Jour. Dairy Res.*, 11: 311-315. 1940.

The presence of haemolytic staphylococci was determined in 92 out of 428 "cow tests." Cases of staphylococcus infection were shown to be missed in mastitis control schemes based on *Str. agalactiae* infection because of suppression of growth on the routine crystal violet blood agar of Edwards. Changes in the composition of the milk were found to accompany staphylococcus infection.

S.T.C.

518. Biennial Reviews of the Progress of Dairy Science. Section E. The Diseases of Dairy Cattle. ANONYMOUS. *Jour. Dairy Res.* 11: 316-350. 1940.

This is a review of recent literature on mastitis, contagious abortion and tuberculosis. 320 references.

S.T.C.

519. The Cellular Content of Milk. Part I. S. B. THOMAS, *Dairy Indus.* 6: 41. 1941.

A survey of the literature on the general subject, with special reference to the leucocyte count and mastitis.

D.V.J.

520. A Simple, Differential Medium for Mastitis Testing. J. G. DAVIS,
Dairy Indus. 6: 38. 1941.

A new differential medium is described and recommended for mastitis work.

Brom-cresol-purple chalk agar

Peptone ¹	3 grams
Yeastrol ²	3 "
Lencol ³	3 "
Lactose ⁴	5 "
Separated milk	10 ml.
Precipitated milk ⁵	10 grams
Agar	15 "
Brom-cresol-purple (0.04 per cent solution)	50 ml.
Tap water	1000 "

Preparation of Medium: Dissolve the peptone, lenco, yeastrol and agar in the tap water, heat to dissolve, filter hot, adjust pH to 6.8 with brom-thymol blue using a comparitor, add the milk, lactose, chalk and brom-cresol-purple solution and fill out with constant shaking, into sterile tubes. Momentarily autoclave to sterilize.

The most valuable feature of this medium is the way the acid-forming colonies dissolve the chalk. In freshly drawn milk these are usually *Str. agalactiae*. In ordinary aged samples a number of types will form zones of clearing in the chalk.

Additional procedures, modifications and interpretation of results are also discussed. D.V.J.

521. The Early Detection of Bovine Mastitis by an Electrometric Method. ERNEST C. McCULLOCH, Div. of Vet. Science, Agr. Exp. Sta., State College of Washington, Pullman, Wash. Jour. Milk Tech. 3: 314-319. 1940.

Early detection and removal of infected animals offers the best means of control. The value of some of the tests used for detecting mastitis is discussed.

A portable electrometric device for detecting mastitis is described. This device is rapid; quarter samples can be made on about 30 cows per hour.

L.H.B.

¹ Any peptone may be used.

² The yeast autolysates on the market are roughly of equal growth promoting value.

³ Any reliable meat extract may be used.

⁴ Dextrose may be substituted to detect those organisms fermenting dextrose but not lactose.

⁵ The chalk must not be heated wet to sterilize before use. It should be sterilized by heating dry for three days at 200° C.

522. A Study of the Mortality Rates of Calves in 335 Herds in England and Wales (Together with Some Limited Observations for Scotland). R. LOVELL, Res. Inst. in Animal Pathology, Royal Vet. College, London: and A. BRADFORD HILL, London School of Hygiene and Tropical Med., London. Jour. Dairy Res., 11: 225-242. 1940.

A total of 27,970 pregnancies was recorded in England and Wales and of these 14.3 per cent failed to produce a calf surviving to the age of 6 months. The authors believe this mortality figures to be an understatement due to the disposal of bull calves often about the first or second week of life, so that their births are recorded but not their later mortality. One thousand, five hundred thirty abortions were reported, or 5.5 per cent of the total pregnancies recorded. Of the 26,440 total births recorded 1,231 or 4.4 per cent were of stillborn animals. Twelve thousand, five hundred forty-four living female calves were born and of these 693 or 5.5 per cent died before the age of 6 months.

Mortality was higher in the first half than in the second half of the year. Stillbirths and abortions were little, if at all influenced by seasonal factors.

Some observations were made on the relation of the observed calf mortality to the methods of calf husbandry. These observations indicate (a) that there is an advantage in allowing the calf to take the colostrum naturally from its mother; (b) that the mortality in these herds was not influenced by the three methods of feeding (suckling, bucket fed with undiluted milk and bucket fed with diluted milk); (c) that mortality was not influenced by the giving or withholding of water to drink.

Nearly half the deaths of female calves took place in the first week of life and three-quarters in the first month. The authors believe coli-bacillosis or white scours to have been an important cause of death. S.T.C.

FEEDS AND FEEDING

523. Weitere Untersuchungen über die Wirkung von Harnstoff und Glykokoll als Eiweisersatz bei der Fütterung von Milchkühen. (Further Experiments Concerning the Use of Urea and Glycine as Protein Substitutes in Feeding Dairy Cows.) K. RICHTER AND W. BIRZER. Ztschr. of Tierernährung und Futtermittelkunde, 4: 59-80. 1940.

Twenty-seven cows were used in this investigation. They were divided into 4 groups, and each group was observed for 70 days with pre- and post-periods of 14 days each. Mixtures containing urea or glycine as a source of protein were consumed by the cows after a period of adjustment. At first it was difficult for the cows to consume these mixtures. As they adjusted to the diets, the glycine mixture was consumed freely and in large quantities.

The first group was fed a normal ration. The other 3 were fed various mixtures containing urea or glycine to balance the nitrogen requirements.

The experiments point to glycine as a possible nitrogen source in feeding cows. Urea as a source of nitrogen could be used to the extent of 50 per cent of the nitrogen supply; with glycine this percentage was 75. Glycine increased the fat percentage in the milk when fed to the cows. Urea as a source of nitrogen for dairy cows decreased milk production. J.C.M.

524. Fütterungsversuche mit Rapsrückständen an Milchkühen. (Feeding Experiments with Rape Products on Cows.) H. BÜNGER, E. FISSMER, AND F. REISING. *Ztschr. f. Tierernährung und Futtermittelkunde*, 4: 183-200. 1940.

This extensive experiment dealt with the use of rape products as rape cake, etc., upon milk yields. Standard oil cake feedings of several kinds and of known influence were used for the controls.

The rape products used included a variety of forms as pressed rape, rape cakes, and cut rape. All were fed in a dry state.

Rape products generally adversely affected milk production when substituted in rations for milk cows. When production of milk was maintained the fat content was reduced.

Rape products must also be free from mustard seed to be usable for cattle feed.

Generally the report is negative in regard to rape products for milk cows. J.C.M.

525. Untersuchungen über die Beeinflussung der Milchfettproduktion durch Mineralstoffe. (Experiments Concerning the Influences of Minerals upon Milk Fat Production.) J. KRIZENECKY, *Tierernährung*, 12: 368-394. 1940.

Calcium, magnesium and phosphorus are associated with milk fat production according to recorded evidence cited by the author.

The author tried commercial products high in minerals with the result that mineral metabolism activated the efficiency of food stuff conversion in the body with ultimately more favorable milk production.

The author enthusiastically proclaims the theories associated with the relationship cited; and states that more factual matter should be obtained to establish reasons for the observations made. J.C.M.

526. The Conservation of Alfalfa, Timothy and Soybean Nutrients as Silages and Hays. A. J. NEWLANDER, H. B. ELLENBERGER, O. M. CAMBURN, AND C. H. JONES, Univ. of Vermont, Burlington, Vt. *Vt. Agr. Expt. Sta. Bul.* 459. 42 pages.

Alfalfa and timothy were made into hay by both sun curing and artificial drying. All three crops were made into silage by the addition of molasses and untreated lots of each were wilted and unwilted. Timothy silages were also made from both wilted and unwilted material treated with phosphoric

acid and by the A.I.V. process. Lots of soybean were likewise treated with phosphoric acid.

Alfalfa. The best silage, pH 4.76, was made from molasses treated material having 40 per cent dry matter and the poorest, pH 5.12, was from untreated material with 25 per cent dry matter which was putrefactive. Calculated recovery of T.D.N. from 100 pounds dry matter ensiled or made into hay was: 48.7 pounds for that wilted and preserved with molasses; 42.2 pounds for that unwilted and not treated; 45.3 for sun cured hay and 51.4 for artificially dried hay.

Timothy. Silages from the lots preserved with molasses and one untreated lot containing 37 per cent dry matter were equally good. The acid treated lots were well preserved but were unpalatable unless ground limestone was added when fed. The poorest lot was untreated and contained 49 per cent dry matter. The highest recovery of T.D.N. per 100 pounds dry matter ensiled was 50.9 pounds for the wilted phosphoric acid treated lot, followed by 50.2 pounds wilted molasses treated and 48.0 pounds for the A.I.V. The lowest was 47.7 pounds for the unwilted untreated. For the sun cured hay the T.D.N. recovery was 53.1 pounds and 53.1 pounds for the artificially dried hay.

Soybeans. Poor silage resulted with or without molasses when the dry matter content was about 25 per cent but when wilted to 31 to 33 per cent dry matter excellent silage resulted. Phosphoric acid preserved the silage well even when the dry matter was 21 to 23 per cent. The recovery of T.D.N. per 100 pounds dry matter of ensiled soybeans averaged 59.5 pounds. There were less losses of nutrients in the molasses treated than in the acid treated silages except for crude protein. W.E.P.

FOOD VALUE OF DAIRY PRODUCTS

527. *Therapeutisch verwendete Sauermilch insbesondere Joghurt und Buttermilch.* (Therapeutic Value of Sourmilk with Special Reference to Yogurt and Buttermilk.) S. HOFFMAN. Schweiz Milch. Ztg. 43: 28 and continued in 44: 215 and in 45: 219. 1940.

This article covers completely the types of organisms normally found in yogurt and buttermilk. The ideas of implantation are discussed; but the theme of the report centers in the value of these drinks because of their protein structure. It is shown that the protein in these milks is readily digestible making them very desirable for children. J.C.M.

528. *Cows' Milk Treated by Base Exchange for Infant Feeding. Metabolism of Calcium, Phosphorus and Nitrogen.* JULIUS H. HESS, HENRY G. PONCHER, HELEN W. WADE, AND JEANETTE C. RICEWASSER. Amer. Jour. Dis. Children, 60: 535-547. 1940.

Unboiled base exchange-treated milk, when fed in the proportion of $1\frac{1}{2}$

ounces per pound of body weight, kept 6 infants below the age of 1 year in positive calcium, phosphorus and nitrogen balance. Essentially the same amounts of calcium, phosphorus, and nitrogen were absorbed and retained from boiled whole milk as from unboiled base exchange-treated milk. The authors conclude that the reduction of calcium and phosphorus content of milk by the process of base exchange does not impair the nutritive value of this milk.

W.H.R.

529. **The Need of Milk in the South.** O. D. ABBOTT, Agr. Exp. Sta., Gainesville, Fla. Jour. Milk Tech., 3: 354-356. 1940.

According to the 1930 U. S. census, the South has 35 per cent of the children under 15 years of age and only 17 per cent of the milk.

In Florida the milk production for the state was sufficient to provide less than 0.4 pint of milk per person per day.

Health records for the State on 10,000 school children show abnormal height-age relationship, vitamin A deficiency and carious teeth. These symptoms indicate a lack of milk in the diet.

L.H.B.

530. **Determining Riboflavin in Dried Milk Products. II. Season Variations.** ROYAL A. SULLIVAN AND EVELYN BLOOM, Kraft Cheese Co., Chicago, Ill. Jour. Milk Tech., 3: 346-349. 1940.

Dried whey (whey obtained after cheesemaking operation) from six drying plants located in five states and having a total annual production of 5,300 tons of dried whey was used as a basis for determining the seasonal variations over a period of two years. Bimonthly samples were tested by a photometric procedure for riboflavin concentration. The method is based upon the extraction of riboflavin with acid-acetone and the distinction of colored impurities by mild oxidation. After filtration, the riboflavin concentration is measured by the determination of light absorption before and after reduction to the leuco form.

The average value of 244 samples was 25.1 micrograms of riboflavin per gram. The mean average for the various seasons was as follows:

Summer	25.6 \pm 0.27	Winter	23.8 \pm 0.39
Autumn	25.6 \pm 0.32	Spring	25.0 \pm 0.29

There was very little difference for any of the seasons. The winter had the lowest value but even this was only seven per cent lower than that for summer.

L.H.B.

531. **The Passage of Carotenoids from Food to Milk in the Cow. The Fate of Lycopene.** A. E. GILLAM, Dept. Chem., Univ. of Manchester, and S. K. KON, Nat. Inst. for Res. in Dairying, Univ. of Reading, England. Jour. Dairy Res., 11: 266-273. 1940.

Evidence was secured that the tomato pigment, lycopene, an isomer of carotene, cannot pass from food into milk in the cow. Three different cows

were fed tomato juice and the resulting milk fats examined separately for lycopene. No indication of the presence of lycopene was detected in the unsaponifiable matter of butterfat when this was subjected to chromatographic adsorption. Lycopene was found in the feces in considerable quantity after tomato feeding. S.T.C.

ICE CREAM

532. **Dextrose and Corn Syrup for Frozen Desserts.** A. C. DAHLBERG AND E. S. PENCZEK. *Ice Cream Field*, 37, No. 3: 36. 1941.

The authors report results obtained with three different types of corn sweeteners used to replace part of the sucrose in ice cream, ices and sherbets. These sweeteners are: (1) an enzyme—converted corn syrup—a new type of liquid corn syrup; (2) corn syrup solids—a dried regular corn syrup; and (3) a hydrated dextrose or corn sugar. They give the relative sweetness of the products studied (on a dry basis) as follows: sucrose 100, enzyme-converted corn syrup 67, corn syrup solids 49, and dextrose 89.

Ice cream mixes were made according to accepted commercial procedures and were prepared to contain 12 per cent milk fat, 10 per cent milk-solids-not-fat, 15 per cent sucrose or its sweetness equivalent, 0.4 per cent gelatin and 0.3 per cent dried egg yolk. The experimental samples were frozen in 1 gallon hand freezers which rotated in cold brine.

Best results were obtained when 25 per cent of the sucrose was replaced with dextrose or corn syrup to give comparable sweetness. Concentrations much greater than this affected adversely the hardness of the ice cream as well as its melting rate.

Titratable acidity of the mixes was not affected by the corn sweeteners but the pH was slightly lowered in the case of the corn syrups. There was no marked effect upon the viscosity or surface tension of fresh mixes as a result of using corn sweeteners, but aged mixes showed a greater viscosity where such sweeteners were used.

The enzyme-converted corn syrup was slightly superior in fresh ice cream and this syrup possessed anti-oxidation properties. They report that both corn syrup and dextrose samples developed an oxidized flavor in 8 weeks of storage, also that in certain cases the color of these samples became bleached.

The development of sandiness was not materially affected by corn sweeteners. Results secured with vanilla and maple-nut ice creams used in these experiments were not constant.

Consumers were unable to identify ice creams on the basis of the presence or absence of corn sweeteners but experts could be trained to do so.

In the case of ices and sherbets sucrose crystallization was prevented by a replacement of 25 per cent of the sucrose with corn sweeteners. W.C.C.

533. Bugaboo of Barriers. PAUL T. TRUITT. *Ice Cream Field*, 37: No. 3: 2. 1941.

The author claims that a trade barrier is "a statute, regulation, or practice which operates or tends to operate to the disadvantage of persons, products, or services coming from sister states to the advantage of local residents." He classifies trade barriers as follows: 1. Those laws which on their face discriminate against out-of-state-enterprise. 2. Those laws which on their face are non-discriminatory but which in practice discriminate against out-of-state enterprise. 3. Laws which apply to residents and non-residents alike which, if encountered in several states, impose a cumulative burden which is a trade barrier. 4. Laws which become trade barriers by virtue of unfair discriminatory administration.

Tabulations now under way will likely show over 3,000 state laws which create or tend to create barriers to the free flow of commerce between states. Forty-one states have enacted laws giving local residents some kind of preferences over non-residents; thirty states limit this preference to state products whereas twenty-four give preference to the home state on printing.

Duplicate inspection requirements for various dairy products have erected barriers between states and in some instances between counties and cities. Examples of such barriers are cited.

There is a reaction on the part of the public against such trade barriers, certain of these laws have been repealed, and other proposed barriers have been defeated. It is suggested that rather than have the Federal Government or the respective State Governments attempt to regulate this problem alone, it would be best to have joint action by the State Governments and the Federal Government, each supporting and reenforcing the others.

The objectives of any program to eliminate trade barriers should be: (1) to prevent the enactment of new trade barrier laws and (2) to obtain the repeal or modification of existing trade barrier laws. W.C.C.

534. Trade Barriers. WILLIAM H. LIST, JR. *The Pa. and N. J. Assoc. Ice Cream Mfrs. Ice Cream Field*, 37, No. 3: 22. 1941.

The author calls attention to Pennsylvania's Act No. 210 dealing with regulation of dairy products. He points out that this law discriminates against dairy products produced in other localities because it does not recognize dairy inspection except under the direction of the Pennsylvania Secretary of Health.

He is in accord with the suggestion of H. A. Ruehe to the effect that "reciprocal acceptance of equivalent quality standard of inspection by health authorities of states, counties and municipalities, would result in a free flow of dairy products from state to state, city to city."

He states that the International Association of Ice Cream Manufacturers

as well as leaders of the industry are in accord with such a plan, but claims that it will be necessary to enlist public opinion in its support in order to accomplish the desired results.

W.C.C.

535. Ice Cream Sales Index for 1940. Statistical and Accounting Bureau. Internatl. Assoc. Ice Cream Mfrs., Washington, D. C. Special Bul. 65. May, 1941.

The increase in sales of wholesale ice cream for 1940 was found to be 2.56 per cent higher than for 1939. This means that in 1940 the largest total gallonage of wholesale ice cream on record was sold. It is estimated that the wholesale gallonage for 1940 was 284,470,672, while the total gallonage was 310,971,115. In Canada the 1940 sales of ice cream were 15.06 per cent higher than in 1939.

A supplement to the bulletin contains statistical data of value, such as: ice cream production departure from 10-year average; average temperature by months; ice cream production for 1939, by months; wholesale ice cream production, 1921 to 1940, inclusive; and the per capita production of ice cream by states, for 1939.

M.J.M.

536. The Use of Whipped Cream in Fancy Forms. B. I. MASUROVSKY, Research Editor. Ice Cream Trade Jour., 37: 36. May, 1941.

With an increase in usage of whipped cream for the decoration of ice cream products it is desirable to know a few facts pertinent to achieving the highest type of product. Rather than use a slightly sweetened 40 per cent butterfat or "heavy" cream, a 30 per cent butterfat cream with 6 to 7 per cent sugar and extra serum solids such as are supplied by condensed skim-milk was used. This was whipped at a temperature of 40° F. and resulted in a product with a better whip and a firmer body. The added sugar is desirable in that it lessens the difference in flavor between the base product and the icing.

Some points for decorating ice cream are: increase the sugar content of whipped cream; have the surface of the base hardened prior to decorating; have smooth edged stencils; use care and rapidity in applying stenciled designs; and be equipped with suitable, convenient facilities for rapid cooling of moulds, cakes, etc.

In decorating ice cream pies, follow the trend usually practised by the pastry chef in designs. Use little or no coloring.

Ice cream cakes should be trimmed with natural or egg color shade cream. Flowers and leaf designs are best made with two tones of some color, i.e., leaves of light green, stalks and the main parts of the plant of a darker green.

The following rules should be given to the consumer as a guide to proper handling of such ice cream desserts:

1. Examine 20 minutes before serving.
2. If too hard, remove the dry ice.
3. Do not touch the dry ice with the bare hands.
4. Wet knife blade with warm water so the blade will cut clean.

W.H.M.

537. **What Is the M.Q. of Your Ice Cream Sales Staff?** H. H. CURNUTT, Steffan Ice and Ice Cream Co., Wichita, Kansas. *Ice Cream Trade Jour.*, 37: 22. April, 1941.

A "M.Q." merchandising quotient test for the ice cream sales staff is presented and analyzed. Ten items are covered in the Quiz. The first 5, each allotted 15 points, deal with the salesman's attitude toward the dealer and the last five, each allotted 5 points, related to the dealer's attitude towards the merchandiser. A score of 100 entitles the salesman to the master merchandiser rating.

To get a perfect score the salesman should answer yes to the following 15 point questions:

1. Do you spend 50 per cent of your time with fountain personnel when calling on dealer?
2. Have you increased sales at 10 per cent of your accounts whose sales were down in the last 6 months?
3. When calling on a dealer do you approximately divide your time in the following proportions? 10 per cent visitation; 30 per cent advertising; 30 per cent sales; and 30 per cent operations.
4. Can you give an accurate report on the consumer trends around each of your dealers?
5. Is it a false statement that, "Some Dealers Cannot Be Merchandised?"

W.H.M.

538. **A 5,000,000-Gallon Delivery System.** JOHN W. BURDAN, Philadelphia Dairy Products Co. *Ice Cream Trade Jour.*, 37: 14. April, 1941.

The Philadelphia Dairy Products Company which distributes 5,000,000 gallons of ice cream annually in six states and which operates 170 trucks and cars, has a series of records and reports which has enabled it to determine the cost of delivering ice cream. Taking all costs into consideration, including drivers' wages, it costs 1.7 cents a quart or 6.8 cents a gallon. Truck depreciation amounts to one cent a mile and truck repairs average 7 to 8 mills. The company estimates the average life of their trucks at 100,000 miles.

The control system gives a complete record of the performance of each truck. It shows charges for drivers' wages, repairs, oil, gas, refrigeration,

maintenance, insurance, tires and tubes, garage rent, tool charges, and accessories such as skid-chains and anti-freeze solution. Savings in oil consumption have been made by installing oil filters on each unit and changing oil only when necessary rather than at a specified mileage. They have found it economical to supply their own mechanics at certain points. Additional savings have been affected by installing a safety program as careless driving may greatly increase the cost of truck operations. W.H.M.

539. Ice Milk. PAUL VASTERLING, Dufold Company, Racine, Wis. *Ice Cream Trade Jour.*, 37: 26. April, 1941.

The author suggests the following formula for Ice Milk: 4 per cent fat, 17 per cent solids-not-fat, 16 per cent sugar, and 0.4 per cent stabilizer, and recommends that the mixture be pasteurized at 160° F. for 30 minutes, homogenized at 2500 pounds pressure, and frozen in a continuous freezer to about the same overrun as ice cream. The material, costing about 17 cents a gallon, will produce a product which usually wholesales for 64 cents a gallon in pint containers. The retailer then sells them for 10 cents each. Most manufacturers limit the 10 cent pint to four flavors.

There is no need to misrepresent the product. It should be labeled "Ice Milk" and the author suggests that the states adopt the proper regulating measures to permit its sale. W.H.M.

540. The Ratio of Total Solids to Butterfat. B. I. MASUROVSKY, Research Editor. *Ice Cream Trade Jour.*, 37: 61. April, 1941.

An optimum ratio of butterfat to total solids-not-fat of 1:2.4 in ice cream is suggested. Such a ratio offers protection against crystallization of milk sugar in the frozen ice cream and makes it possible to obtain 100 per cent overrun and still comply with a requirement of 1.6 pounds of food solid per gallon of ice cream. Ice cream weight charts are presented which show the ratio of butterfat to total solids-not-fat in mixes with varying percentages of total solids.

A fixed standard ratio of 1:2.4 will not apply to every type of ice cream; however, it can be varied to meet local conditions. W.H.M.

541. Merchandising Innovations. ANONYMOUS. *Ice Cream Trade Jour.*, 37: 29. April, 1941.

Unorthodox, yet result producing, is the sale of ice cream by weight to the customers in their own containers practised by the Queen City Dairy of Cumberland, Md. A 13 per cent fat product that weighs 4½ pounds per gallon is sold for 20 cents a pound, hand-dipped. The salesgirls are schooled in explaining that such is practised to assure the same amount of ice cream to every customer.

Other innovations of the Queen City plant is the putting on a "show" for the customers' benefit when serving any product, whether it be a sundae or a malted milk. "Make the customer see his money's worth" is the philosophy practised by the sales girls. Malts are made by pouring a full half-pint of milk into the cup, adding 2 "slices" of ice cream and a dash of whipped cream—all of which looks large. Dishes are served with 2 "slices" of ice cream rather than one with the idea of creating the effect of quantity as well as quality.

Important, too, is the use of a high-type sales girl who knows how to learn her job and will readily fit into the mould of sales procedures of the store already established.

W.H.M.

542. Milk and Ice Cream Drinks. LOUIS D. JONES. *Ice Cream Trade Jour.*, 37: 30. May, 1941.

Since the mixed milk and ice cream drink represents the major channel of outlet of ice cream at the soda fountain, the wholesaler of ice cream as well as the retailer should know what the public desires. A government survey shows that 26.88 cents of the retail ice cream dollar was spent for mixed milk and ice cream drink; 24.55 cents for carry-outs; 22.78 cents for sundaes; 19.08 cents for sodas; and 6.71 cents for novelties. The finding of a national research bureau was that more than 50 million dollars was spent for milk and ice cream drinks than was spent for all other soft drink beverages as soda, carbonated fruit juices, and colas sold over the soda fountain.

The prerequisite of a good milk and ice cream mixed drink is taste; and the taste is dependent upon the quality of ingredients, which must be high, and the smoothness of mixing of the ingredients. Purpose of mixing is to blend the flavor constituents and to aerate or fluff the drink for the desirable smoothness. Proper mixing is dependent upon the products used and the type of finished product desired. A well designed mixer will not beat the overrun out of the ice cream at the same time incorporating overrun into the milk and blending the flavors together.

Milk at 32° F. will whip to a 90 per cent overrun, while at 40° F. the overrun is only 82 per cent or a decrease of 13 per cent, with another 8° F. increase to 48° F. the overrun is 55 per cent. Thus it can be seen the importance of maintaining proper temperatures of ingredients of the mixed drink.

W.H.M.

543. Payload Progress. VINCENT M. RABUFFO. *Ice Cream Trade Jour.*, 37: 10. April, 1941.

The delivery costs of ice cream have been reduced by increasing the size of the pay load and by reducing the size of the truck chassis. Dry ice and

mechanically refrigerated truck bodies have lowered refrigeration costs and prolonged the life of delivery equipment over what it was when ice and salt were used. By reducing the size of the truck there has been a further saving in the cost of a truck license. Today a one and one-half ton truck can haul 1,000 gallons of ice cream at a per day cost of less than \$5.00 or a mileage cost of less than \$0.07 which is one-fourth to one-third the cost which prevailed a few years ago.

W.H.M.

MILK

544. Der Einfluss des Gefrierens auf entrahmte Milch. (The Influence of Freezing upon Creamed Milk.) H. OSTERMANN AND E. PREVOT. Deut. Molkerei Ztg., 25: 51-52. 1940.

The freezing point, specific gravity, degree of acidity, and composition of creamed milk frozen solid were observed. The main findings pointed out the necessity of completely thawing and mixing such milk before use. Although partial thawing appeared to produce uniform portions of upper and lower milk, actual analysis showed wide variation in composition due to a lack of completely thawing before using.

J.C.M.

545. Ascorbic Acid Content of Cow's Milk at Various Stages at Lactation. ARTHUR D. HOLMES, FRANCIS TRIPP, E. A. WOELFFER, AND G. H. SATTERFIELD. Amer. Jour. Dis. Children, 60: 1025-1030. 1940.

Three hundred fifteen samples of Guernsey milk, and three hundred thirty-seven samples of Holstein milk were assayed for ascorbic acid content. Of these samples, 75 to 90 per cent for each breed, respectively, were of milk produced during the second to eleventh months of lactation.

The ascorbic acid content of both Guernsey and Holstein milk rose rapidly during the first two months of lactation. From the second to the eleventh months, a slight decrease was observed. Average monthly values for ascorbic acid in Guernsey milk varied from 19.39 mg/1000 cc. for the eighth month to 21.49 mg. for the second month. For Holstein milk, the range in variation was from 15.68 mg. for the first month to 18.85 mg. for the fifth month.

W.H.R.

546. How Equipment Selection Influences Plant Design and Operation. H. McNABB, Sheffield Farms Co., New York, N. Y. The Assoc. Bul., Internatl. Assoc. Milk Dealers, 33rd Year, 15: 393-398. March, 1941.

The arrangement of plants to allow milk to flow by gravity from one piece of equipment to another, each on a different floor level, has resulted in many extra steps in getting from one piece of equipment to another and has

made it difficult to remodel or adapt the plant for other uses. Support columns and the resulting loft type building efficiently accommodates various operations with low cost and flexibility. In another plant, by leaving out parts of the second floor, the equipment appeared to be on balconies and an effect of spaciousness was obtained. The remainder of the second floor can be filled in as needed. Floors need to carry heavier loads than formerly and attention is called to the fact that certain 5500 gallon tanks do not have the load evenly distributed among the legs. An instance is given of a 5 leg buttermilk tank, where the center leg carried half the load or 6,250 lbs., the remaining weight being divided between the other 4 legs.

Locating floor drains near columns has left the center of the floor thicker and stronger and obviates having drains under equipment. At each corner of every column a 6-inch floor sleeve is installed to obviate cutting the floor for pipes later. Also all vertical pipes have plugged tees just below the floor slab for future connections.

Additional electrical switches are installed for future use and heavy duty elevators to move machinery. Triple doors and some masonry wall sections not bonded into the remainder of the wall make moving large machines easier.

E.F.G.

547. **A Proposed New Common Sense Milk Can.** H. A. TREBLER, Seal-test, Inc., Baltimore, Maryland. The Assoc. Bul., Internatl. Assoc. Milk Dealers, 33rd Year, 15: 393-398. March, 1941.

This can is seamless, 16 gauge metal with an 8½" diameter straight neck and no pouring lip. An Umbrella type cover is used. The breast of the can tapers down far enough so that all of the inside can surface may be seen. The handle is the upright stiff metal type, placed a little lower down than usual. The bottom has large drain holes, permitting alkali solution and water to drain off rapidly.

The advantages of the can are that it will drain about 30 per cent faster and leaves 2 ounces less milk in each can when draining 14 cans per minute. It is cleaned easier with a hydraulic washer and the cost of manufacture of the can should be somewhat less.

E.F.G.

548. **Bacteriological Aspects of Farm Milk Cooling.** T. G. ANDERSON. Pa. Agr. Expt. Sta. Bul. 404. 1941.

In this study of farm milk cooling, the use of well water was compared in effectiveness with electrical refrigeration. The results obtained by taking samples of milk at three levels in a 10-gallon can showed positive necessity for quick and complete cooling. The difference in numbers of bacteria before and after cooling for 12 hours emphasized the importance of low temperatures. The rise in temperature of cooled milk during transportation indicated the need of proper protection during hauling to receiving plants.

Author's Abstract

549. **Testing the Sterility of Bottles.** H. BARKWORTH, Dairy Indus., 6: 35. 1941.

The technique proposed by Mattick and Hoy (a 20 cc. rinse) was found to be superior to the conventional 90 cc. rinse. The proposed standard of 200 bacteria per bottle can be maintained commercially. When this standard is enforced a coliform test gives no additional information and is superfluous.

D.V.J.

550. **Can Cleaning and Sterilization.** A. L. PROVAN AND A. R. TREBLE. Dairy Indus., 6: 5. 1941.

With the support of experimental bacteriological data the authors demonstrate the importance of returning clean dry milk cans to the farm. Mechanical and hand can washing are discussed and control measures to insure efficient sterilization are suggested.

D.V.J.

551. **Practical Plant Operating Problems.** HANS EDEL, Gehl's Guernsey Farms, Milwaukee, Wis. The Assoc. Bul., Internatl. Assoc. Milk Dealers, 33rd year, 15: 404-408. Mar., 1941.

Refrigeration efficiency is obtained in a number of ways, among which are low discharge pressure by means of frequent purging and draining oil from the system. Frequent defrosting promotes efficiency. A booster pump on the suction line may help. Frosted cylinder walls are an indication of inefficient operation.

It is recommended that scale be removed from a bottle washer by means of 8-hour treatment with a 6 per cent hydrochloric acid solution plus perhaps 3 to 6 ounces of inhibitor per carboy of acid to prevent corrosion.

Figures are given to show 4.85 pounds of washing powder per 1000 bottles were used before descaling and 1.2 pounds of washing powder were used per 1000 bottles after descaling the washer.

E.F.G.

552. **Dairy Herd Management Practices Affecting the Quality of Milk.** A. C. RAGSDALE, Dairy Husbandry Dept., Univ. of Missouri. Jour. Milk Tech., 3: 350-353. 1940.

Practices affecting the quality of milk are discussed together with control methods which may usually be found effective.

L.H.B.

553. **Application of the Resazurin Test in Determining the Quality of Pasteurized Cream.** W. H. CHILSON AND M. A. COLLINS, United Farmers Cooperative Creamery Assoc., Inc., Boston, Mass. Jour. Milk Tech., 3: 334-340. 1940.

Using 1.0 ml. of 0.005 per cent resazurin solution to 10.0 ml. of cream and incubating at 98° F. until a pronounced pink color was obtained would

give reliable information within a few hours after pasteurization of the quality of the cream. A reduction time of six hours or longer (to pronounced pink color) indicated fine quality in pasteurized 20 or 40 per cent cream. Such cream will generally show not more than 40,000 bacteria per ml. when aged four days at 40–45° F.

A reduction time of over five hours usually indicates a standard plate count of less than 100,000 bacteria per ml. in nine of ten samples and of 40,000 or less in seven of ten samples.

When the test is applied to pasteurized cream, which has been stored at temperatures of 40° F. or lower for four or more days, it could not always be depended upon to indicate high counts.

There was a much closer agreement between the resazurin test and the standard plate count than between the rise in acidity upon incubation at 72° F. for 15 hours and the standard agar plate count. L.H.B.

554. A Modified Resazurin Test for the More Accurate Estimation of Milk Quality. C. K. JOHNS AND R. K. HOWSON, Dominion Dept. of Agr., Ottawa, Canada. *Jour. Milk Tech.*, 3: 320–325. 1940.

A study was made of 279 samples of market milk to determine a simple method of routine grading which would combine the sensitivity of the "one-hour" test for abnormal milk with the greater accuracy of the resazurin "pink" end point for bacteria. This was found to be color number 8, described as P 7/4 according to the Munsell system of color notation.

By the use of this standard, milk may be classified into four grades in three hours time. All samples showing a color number in excess of 8 at 1 hour would go into the 4th grade. Those showing such a change at 2 hours would go in class 3; those showing the change at 3 hours would go in class 2; and those not showing such a change in 3 hours would go in class 1.

The use of a daylight lamp is important in making color comparisons. A 15-watt (General Electric) Mazda fluorescent daylight lamp with a neutral grey background was found superior to other types of daylight lamps tried. L.H.B.

PHYSIOLOGY

555. Influence of Thyroidectomy on Fat Deposition in the Rat. EATON M. MACKAY AND JAMES W. SHERRILL. *Endocrinology*, 28: 518. 1941.

Thyroidectomy in the adult male albino rat resulted in a marked reduction in the amount of body fat in comparison with unoperated controls of the same age when they were killed 10 months later. The thyroidectomized rats, in spite of their low fat content, neither appeared less plump nor did they weigh appreciably less than the controls. The inclusion of active thyroid substance in the diet produced the usual decrease in body fat.

R.P.R.

556. Qualitative Progesterone Assay of Pregnant Cattle AP and Extracts Having Mammary Growth Activity. J. J. TRENTIN, J. P. MIXNER, A. A. LEWIS, AND C. W. TURNER. Soc. Exptl. Biol. and Med. Proc., 46: 440. 1941.

Fresh pregnant cattle pituitary tissue in amounts which stimulated growth of the lobule-alveolar system in spayed mice was found to contain insufficient progesterone to produce a positive response by the sensitive McGinty technic. Lipid extracts of the AP which stimulated duct growth in the male mouse were also found to be negative for progesterone. The authors believe that these observations indicate that neither the mammogenic duct nor lobule-alveolar effects of the AP are due to the presence of progesterone.

R.P.R.

557. The Assay of Prolactin by Means of the Pigeon Crop-Gland Response. S. J. FOLLEY, F. J. DYER, AND K. H. COWARD. Jour. Endocrinology 2:179. 1940.

The results of lactogen assays by the pigeon crop-weight method were analyzed statistically. For a given dose of hormone there was a positive correlation between crop-weight and body-weight. A sigmoid curve was obtained when either absolute crop-weight or crop-weight expressed as a percentage of body-weight was plotted against hormone dose. The relationship between either of these quantities and log dose was approximately rectilinear for total doses from 3 to 18 I.U. The limits of accuracy of the assay method compared favorably with other biological assays. Temperatures above 15° C. decreased the stimulation of the crop-glands by lactogen while light had no influence upon the response. A subjective estimate of the crop-milk of pigeons injected with lactogen was suggested as the basis of an approximate assay method. It was suggested that the following conditions should be observed in order to attain a satisfactory degree of accuracy in lactogen assays: 1. Each group should consist of 15-20 birds; 2. All birds should be kept at the same temperature, preferably near 15° C.; 3. The body-weights of the birds should lie within the limits of 260 to 360 grams; 4. Hormone injections should be made subcutaneously; 5. Simultaneous comparison between the unknown and the standard preparation of lactogen should be made; and 6. Calculations should be made on the basis of crop-weights expressed as percentages of body-weights.

R.P.R.

558. Prolactin as a Specific Lactogenic Hormone. S. J. FOLLEY AND F. G. YOUNG. Lancet, 240: 380-381. 1941.

A review of the literature with 32 references.

J.F.C.

559. Effects of Oestrogens on Lactation. S. J. FOLLEY, Nat. Inst. for Res. in Dairying, Univ. of Reading. Lancet, 240: 40-41. 1941.

A review of the literature with 33 references.

J.F.C.

560. Further Experiments on the Continued Treatment of Lactating Cows with Anterior Pituitary Extracts. S. J. FOLLEY AND F. G. YOUNG. Jour. Endocrinology, 2: 226. 1940.

The injection of a crude anterior pituitary extract subcutaneously on 11 alternate days into cows in declining lactation increased the average daily milk production during the injection period to 16 per cent above that expected in the absence of treatment. Similar injections of a lactogen preparation increased the average milk yield to only 5 per cent above that expected. In both instances milk production declined in magnitude towards the end of the injection period, but no evidence was found for the presence of antilactogen activity in the serum of the cows at that time. The injection of a pituitary preparation having glycotropic activity but no detectable lactogen had no obvious influence on the milk production of cows in declining lactation. The content of fat or of non-fatty solids in the milk was not affected by the pituitary injections.

R.P.R.

MISCELLANEOUS

561. A Study of the Sensitiveness of Prospective Food Judges to the Primary Tastes. DARLINE KNOWLES AND P. E. JOHNSON, North Dakota Agr. Exp. Sta., Fargo, N. D. Food Res., 6, No. 1: 207. Mar.-Apr., 1941.

In a study using 19 men and 18 women it was found that individuals varied widely in their ability to detect low concentrations of substances producing the four primary tastes. There was no significant difference between the ability of the men and that of the women. Of the 37 people participating five were rated excellent, four good and three fair. No correlation was noticed between judging ability and age, experience in judging or smoking. The authors state that results show the necessity for testing the tasting ability of individuals previous to making selections for a panel of judges in the examination of foods.

F.J.D.

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PUBLICATIONS AND ABSTRACTORS

EDITORS

Dahle, C. D., Dahlberg, A. C., Elliker, P. R., Petersen, W. E.,
Tracy, P. H. and Weckel, K. G.

ABSTRACTORS

Anderson, E. O.	Dorsey, L. M.	Josephson, D. V.	Reece, Ralph P.
Archibald, J. G.	Downs, P. A.		Riddell, W. H.
	Erb, J. H.	Knight, D.	Ritter, W.
Babcock, C. J.	Ely, Fordyce		Stark, C. N.
Berggren, Ruth E.	Espe, D. L.	Lucas, P. S.	Stebnitz, V. C.
Brueckner, H. J.	Frazier, W. C.	Lush, J. L.	
Burgwald, L. H.		Mack, M. J.	Thomsen, L. C.
Bushnell, L. D.	Garrett, O. F.	Macy, H.	Trout, G. M.
	Glick, D. P.	Marquardt, J. C.	
Cole, W. C.	Goss, E. F.	Martin, W. H.	Webb, B. H.
Cone, J. F.	Hansen, Arne	Mueller, W. S.	Weckel, K. G.
Corbett, W. J.	Huffman, C. F.		White, G. C.
Coulter, S. T.		Price, W. V.	Yale, M. W.
Doan, F. J.	Irvine, O. R.		

JOURNALS

American Butter Review	Journal of Genetics
American Milk Review	Journal of Infectious Diseases
American Journal of Diseases of Children	Journal of Milk Technology
American Journal of Physiology	Journal of Nutrition
American Journal of Public Health	Journal of Pathology and Bacteriology
Archives of Pediatrics	Journal of Physical Chemistry
	Journal of Physiology
Biochemical Journal	Kaeseindustrie
Biochemische Zeitschrift	Kolloid-Zeitschrift
	Lancet
Canadian Dairy and Ice Cream Journal	Le Lait
Canadian Public Health Journal	
Certified Milk	Milchwirtschaftliche Forschungen
Cornell Veterinarian	Milchwirtschaftliche Zeitung
	Milk Dealer
Dairy Industries	Milk Industry
Dairy World	Milk Plant Monthly
Deutsche Molkerei Zeitung	Molkerei Zeitung
	National Butter and Cheese Journal
Endocrinology	
	Oil and Soap
Food Industries	Pacific Dairy Review
Food Manufacture	Proceedings of Society of Animal Production
Food Research	Proceedings of Society of Experimental Biology and Medicine
Ice and Refrigeration	Refrigerating Engineering
Ice Cream Field	Scientific Agriculture
Ice Cream Review	
Ice Cream Trade Journal	Tierernahrung
Industrial and Engineering Chemistry	Tierzüchter
	Zeitschrift für Infektionskrankheiten Parasitäre Krankheiten und Hygiene der Haustiere
Journal of Agricultural Research	Zeitschrift für Physikalische Chemie, Abt. A and B
Journal of Agricultural Science	Zeitschrift für Untersuchung der Lebensmittel
Journal of American Veterinary Medical Association	Zeitschrift für Züchtung, Reihe B. Tierzucht- und Zuchtungsbiologie
Journal of Bacteriology	Zentralblatt für Bacteriologie
Journal of Biological Chemistry	Züchtungskunde
Journal of Dairy Research	
Journal of Dairy Science	
Journal of Endocrinology	
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Journal of General Physiology	
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SPECIAL PUBLICATIONS

Federal Dairying and Bacteriological Establishment, Liebfeld, Berne, Switzerland	Prussian Dairy Research Institute, Kiel, Germany
International Association of Ice Cream Manufacturers	State Agricultural Colleges and Experiment Stations
International Association of Milk Dealers	
National Institute for Research in Dairying, Reading, England	The Royal Technical College, Copenhagen, Denmark
New York Association of Dairy and Milk Inspectors	United States Department of Agriculture

ABSTRACTS OF LITERATURE

ADVANCE ABSTRACTS OF REPORTS ACCEPTED FOR PUBLICATION IN THE JOURNAL OF DAIRY SCIENCE

562. Some Ocular Changes and Deficiency Manifestations in Mature Cows Fed a Ration Deficient in Vitamin A. L. A. MOORE, Michigan State College, East Lansing.

Mature cows fed a vitamin A deficient ration failed to develop blindness due to constriction of the optic nerve such as has been reported in calves. A definite papilledema failed to develop in two out of six animals fed the deficient ration. However, once the papilledema develops it takes considerable time for it to recede. They did develop nyctalopia, incoordination and an edema of the legs. The tapetum nigrum and lucidum developed a mottled appearance. When the plasma carotene values receded to a 0.2 to 0.5 microgram level per ml., deficiency symptoms usually followed in a short period of time. The fat of a Guernsey cow which died with symptoms of vitamin A deficiency showed the presence of a pigment which was most likely carotene since it was epiphasic between petroleum ether and 92 per cent methyl alcohol.

563. Preservation of Bovine Spermatozoa in Yolk-Citrate Diluent and Field Results from Its Use. G. W. SALISBURY, H. K. FULLER, AND E. L. WILLETT, Dept. Animal Husbandry, Cornell University, Ithaca, N. Y.

Investigation had earlier shown that the buffer capacity of bull semen was primarily due to citrates, phosphates, and carbonates. In an endeavor to develop a buffer solution to be used with egg yolk for semen studies having characteristics similar to the buffers of bull semen plasma, it was noted that a solution containing a high proportion of sodium citrate was effective in clearing the egg yolk. When using the microscope to examine a sample of semen diluted with this mixture one could readily discern the individual sperm; on the other hand, when using the yolk-phosphate diluent it was necessary to further dilute the mixture with a clear diluter before the individual sperm could be distinguished. This property was lost when the proportion of citrate was reduced.

A mixture of approximately one-half fresh egg yolk and one-half of an M/15 solution of sodium citrate produced a diluent which gave as good results as any other studied. When compared with the yolk-phosphate diluent the yolk-citrate mixture preserved the motility of spermatozoa at as satisfactory a level for two and four days of storage. For periods of storage of 6, 8, and 10 days it was superior to the yolk-phosphate diluent.

In a controlled field experiment to determine the effectiveness of the yolk-

citrate diluent in preserving the fertility of spermatozoa over periods of storage up to 5 days it was found that 178 services were required for 118 conceptions for semen stored in the yolk-citrate diluent. When the yolk-phosphate diluent was used, 193 services were required for 126 conceptions over the same period of storage for semen from the same bulls. No significant differences were noted in the rate of conception over the total storage period nor for any portion of the total storage period between the two diluters used.

564. Oxidized Flavor in Milk. X. The Effect of Feeding Potassium Iodide Supplements to Dairy Cows on the Carotene Content of the Butter Fat and on the Ascorbic Acid Content of the Milk and the Relationship to Metal-Induced Oxidized Flavor. W. CARSON BROWN, A. H. VANLANDINGHAM, AND CHAS. E. WEAKLEY, JR., West Virginia Agr. Expt. Sta., Morgantown.

A study of oxidized flavor in milk produced during two periods in which potassium iodide was supplemented to the normal ration revealed no relationship to the development of oxidized flavor. Potassium iodide was supplemented at the rate of 5 grams daily for a period of 14 days to five animals. In the second trial the supplement was repeated after a seven-day readjustment period. Two animals were used in the second trial.

As a result of these trials the following conclusions were drawn:

1. The feeding of 5 grams daily of potassium iodide for 14 days lowered to a marked degree the percentage of ascorbic acid secreted in the milk, but had no noticeable effect on the level of the carotene content of the milk.

2. The decrease in the ascorbic acid content of the milk did not produce a corresponding increase in the intensity of the metal-induced oxidized flavor.

3. From these results it appears that the level of the ascorbic acid in the milk may not be as great a factor in the production of milk with low susceptibility to oxidized flavor as was formerly believed.

565. An Analysis of the Relationship between the Curd Tension and the Curd Surface Area of Milk. ARNOLD B. STORRS, American Seal-Kap Corp., Long Island City, N. Y.

A statistical analysis of the relationship between the curd tension and the curd surface area of milk was made. No significant relationship was found within individual types of commercially modified milks. Within mixed groups of various types of modified milk the relationship between curd tension and curd surface area was found to be of variable significance and seemed to depend upon the types of milk included in the particular group.

The conclusion is offered that curd tension and curd surface area are

independent characteristics of milk and that each may be influenced or determined by factors not closely related.

566. The Effect of Hydrogenation on the Nutritive Value of the Fatty Acid Fractions of Butter Fat and of Certain Vegetable Oils.

R. K. BOUTWELL, R. P. GEYER, C. A. ELVEHJEM, AND E. B. HART,
Dept. Biochem., Univ. of Wisconsin, Madison.

The superior growth-promoting property of butter fat as compared to certain vegetable oils is probably due to a saturated compound; apparently a long chain saturated fatty acid (or acids) present in small amounts in butter fat is responsible for these properties of butter fat.

The unsaturated fraction of butter fat is relatively rich in an unsaturated form of this compound which by hydrogenation may readily be converted to the active compound.

Certain vegetable oils as corn oil, coconut oil, cottonseed oil and soybean oil apparently do not contain the unsaturated form of this compound. Hydrogenation of these vegetable oils did not improve their nutritive value when incorporated into skimmed milk.

567. The Effect of Vitamin A and Certain Members of the B-Complex upon Calf Scours.

PAUL H. PHILLIPS, NORMAN S. LUNDQUIST, AND
PAUL D. BOYER, Depts. of Biochem. and Dairy Husbandry, Univ.
of Wisconsin, Madison.

The effect of vitamin A and certain members of the B complex on calf scours has been studied with these results. These studies indicate that the calf diarrhea encountered was largely nutritional in origin. The administration of high vitamin A potency shark liver oil and certain members of the vitamin B complex eliminated the diarrhea and the resulting mortality from pneumonia. Preliminary evidence would suggest that nicotinic and pantothenic acids may be the factors of the B complex which were lacking.

New-born calves were found to be amply fortified with ascorbic acid but they were uniformly deficient in vitamin A. The ingestion of colostrum milk rich in vitamin A quickly brought about normal blood plasma levels. The ration of the dam influenced to some extent the amount of vitamin A found in the blood plasma of the new-born calf. Winter rations tend to reduce it while rations with ample carotene or fortified with vitamin A tend to raise it. Low ascorbic acid values in the blood plasma were increased by feeding shark liver oil rich in vitamin A.

568. Factors Affecting the Gas Content of Milk. C. I. NOLL AND G. C. SUPPLEE, Borden Biol. and Chem. Res. Labs., Bainbridge, N. Y.

A quantitative study has been made of the dissolved gases in milk as affected by light, heat, vacuum, displacement by other gases and processing,

in order to observe the general principles governing the gas content of milk. Particular attention has been given to the oxygen content of milk and the study of methods for its removal.

Data are submitted showing that, within the limitations of the experimental procedures used, the oxygen content of milk is primarily a function of the partial pressure of the oxygen over the solution and the temperature of the solution. This is in agreement with the accepted laws of solutions of gases in liquids.

Quantitative experimental data are given showing the gas content of milk at various stages in several pasteurization processes, the loss of gases during the heating and their subsequent reabsorption on exposure to air during cooling.

The degree to which the oxygen content of milk can be lowered by heat (below boiling temperature), vacuum, displacement with other gases, light and light in the presence of added ascorbic acid is presented. The application of these data to the development of methods for the deoxygenation of milk is discussed with appropriate experimental evidence.

The correlated data show that if the dissolved oxygen in milk is completely removed, the vitamin C of fluid or processed milk is stable, notwithstanding subsequent heat treatment or exposure to light. Such factors as heat, exposure to light, the presence of copper, etc., appear to be secondary catalytic influences affecting the rate of destruction of vitamin C only if dissolved oxygen is present.

569. The Lethal Effectiveness of Ultraviolet Rays Applied to Milk.

G. C. SUPPLEE, G. E. FLANIGAN, AND O. G. JENSEN, Borden Biol. and Chem. Res. Labs., Bainbridge, N. Y.

The lethal effectiveness of ultraviolet radiation is well known, but available evidence concerning the degree of destruction of bacteria in milk under conditions which are adaptable for practical use, is very meager. The results from studies with commercial milk extending over a period of some years, have revealed the merits and limitations of this bactericidal principle as applied to milk wherein certain improvements in the experimental technique were employed.

By irradiating smooth flowing milk films of known characteristics and using appropriate spectral quality and intensity of the incident radiation, a reduction in bacteria count of average raw milk of 95 to 98% was obtained, with substantial regularity. This reduction may be accomplished without development of adverse flavor and odor within an exposure period of about seven to eight seconds or less. The spectral characteristics and the intensity of the radiations and method of application were found to be more significant in obtaining a consistent high percentage reduction, than variations in the resistance of the organisms comprising the usual milk flora.

Sub-lethal applications of ultraviolet energy of which a predominant proportion consisted of short radiation (2200–2300 Å but with none of the 2537 Å line) gave irregular results with evidence that such radiation may actually increase the bacteria count of milk under given conditions. Whether the increase in plate counts was due to a dispersal of clumps or to a stimulating effect on individual organisms is a matter of conjecture.

Irradiation at elevated temperatures, or simultaneous irradiation during elevation of the temperature by electrical heating of the flowing film, did not significantly enhance the lethal effectiveness of the ultraviolet energy; such method of treatment tends to develop a characteristic irradiation flavor.

Percentage reduction curves obtained with an experimental flowing film electric pasteurizer wherein the temperature may be raised to any desired degree within a period of about 0.8 second are compared with percentage reduction curves obtained by ultraviolet radiation under varying conditions of treatment. The data illustrate comparatively, the bactericidal effectiveness of both forms of energy applied to milk under conditions potentially adaptable for other than laboratory demonstration, and wherein the time element is reduced substantially to an irreducible minimum.

570. The Reliability of the Room Temperature Holding Test as an Index to the Keeping Quality of Butter. D. H. JACOBSEN, C. C. TOTMAN, AND T. A. EVANS, South Dakota State College, Brookings.

About 78 samples of butter were used in a study of the holding test. Scoring contest butter from entries of 3 different years and from 28 South Dakota creameries was used. Fresh scores ranged from 89 to 94 with the largest percentage ranging from 90 to 93. The salt content varied from 0.5 to 3.0 per cent and the average was 1.8.

The holding test conditions were 7 days at 70° F. The butter was scored when fresh and again at 7 days at 70° F. and after 30 days at 40° F. Samples losing most in score in the holding test, lost less in 30 days at 40° F. Samples losing least in score in the holding test, lost more in 1 month at 40° F. but only slightly more. Reference is made here to averages; several exceptions were noted. In most cases, scores under the 2 holding conditions show considerable correlation.

Flavor criticisms of the butter after holding indicate that greater bacteriological changes took place at 70° F. and that chemical changes were greater at 40° F. Yeast and mold counts on fresh butter showed no relation to keeping quality.

BOOK REVIEWS

571. Principles of Dairying. HENRY F. JUDKINS AND MERRILL J. MACK. 315 pp. Published by Wiley. 1941.

The third edition of this book in elementary dairying has been revised

by Professor Merrill J. Mack of Massachusetts State College. The book treats the subject largely from the standpoint of handling of milk and milk products.

The table of contents remains practically the same as the second edition (Judkins and Smith) with the exception of a new chapter added entitled "Quality Tests for Milk," to replace the chapter on "Acidity and Its Relation to Dairy Products." This new chapter contains considerable material not included in previous editions.

Much new material has been added to bring the book up-to-date. New illustrations, tables, problems and references have been added. Questions, problems and suggested practicums are given after each chapter. The chapters on "Properties of Milk," "Testing of Milk and Milk Products" and "Food Value" have been enlarged considerably and include much new information. The nineteen chapters cover the following subjects:

- The General Scope of the Dairy Industry
- The Secretion of Milk
- The Composition and Properties of Milk
- Factors Affecting the Composition of Milk, Particularly the Butterfat Content
- The Sampling of Milk and Cream
- The Babcock Test for Whole Milk
- The Babcock Test for Milk Products
- Testing Milk for Total Solids
- The Bacteriology of Milk
- Quality Tests for Milk
- Keeping Milk and Butterfat Records
- Essentials in the Production and Handling of Market Milk on the Farm
- Market Milk from Farm to Consumer
- The Separation of Cream
- Butter Making
- Ice Cream Making
- Cheese Making
- Miscellaneous Dairy Products
- The Food Value of Milk and Its Products.

C.D.D.

572. **Brucellosis (Undulant Fever) Clinical and Subclinical.** HAROLD J. HARRIS. Paul B. Hoeber, Inc., New York. 1941. 286 pp., illustrated. Price, \$5.50.

This excellent monograph on brucellosis was written primarily for the medical profession. However, it also provides information of definite interest to all who at some time or other might be affected by brucellosis or be responsible in any way for conditions or situations influencing its spread or transmittance.

The author has included a historical review as well as chapters on the etiology, epidemiology, pathology, symptomatology, diagnosis, prognosis,

treatment and prophylaxis of the disease. The numerous case histories with photographs and detailed descriptions of symptoms, diagnosis and reaction to treatment should prove highly useful to practicing physicians whose experience with this disease has thus far been limited. The author repeatedly emphasizes, as have recent investigators in this field, that while acute brucellosis frequently is recognized, the more common chronic form is in many cases never reported to a physician and altogether too often diagnosed incorrectly. According to the author, "There is no infection, except syphilis, that masquerades under so many guises as does brucellosis." He agrees with Levine and associates in their statement that "When brucellosis becomes chronic the one constant symptom is weakness; fever may not be present at all. The symptoms are confused with neurasthenia because there is exhaustion, insomnia, irritability and complaint of aches and pains for which no objective signs can be found."

Incidence figures of Gould and Huddleson reported in 1938 are interpreted as follows: "While an actual census made throughout the United States on a given date would probably reveal 120,000 persons clinically ill with the disease, any of the 12,000,000 infected persons may be added to that census as their infections wax and wane."

All public health officials and particularly dairy and meat sanitarians would profit by reading the chapters entitled respectively Epidemiology and Prophylaxis-pasteurization as well as the medico-legal aspect treated in the Addenda. In the chapter on prophylaxis, the author presents what he considers a desirable two point program to combat this disease: "(1) Pasteurization of all milk (the absolute prohibition of the sale of raw milk from any herd, no matter what its history and its record of laboratory tests). (2) A nation-wide campaign to destroy all infected cattle, sheep, goats, pigs, horses and all farm or domestic animals known to harbor the disease."

This two-point program is proposed to protect those individuals who habitually or occasionally drink infected raw milk and those whose occupations expose them to virulent organisms. The latter group includes stockyard and slaughter-house employees, butchers, meat dealers, chefs, farmers, dairymen, veterinary surgeons, and laboratory workers. Obstacles involved in and benefits to be attained from such a program are discussed. P.R.E.

573. Annual Review of Biochemistry. Vol. 10, 1941. Published by Annual Reviews, Inc., Stanford University P. O., Calif. 692 pp. \$5.00.

The **Annual Review of Biochemistry** is a volume consisting of chapters on biochemical subjects written by various individuals at the request and invitation of an editorial board. At times, certain subjects of broad aspect are discussed in consecutive years by various writers, thus permitting pre-

sentation of more than one viewpoint or approach in discussion. The volume is definitely by and for those engaged in educational, research and supervisory activities, although much of the material is of value to individuals having a "lay" knowledge and seeking review material. It is the intent of the Annual Reviews to consolidate the newer knowledge of a subject made available over a relatively recent period, say one, or two or three years. In this respect the volume is an authoritative review and will be of immediate value to the worker desiring to keep informed of the progress in the biochemical sciences. Volume 10 contains 24 chapters, plus a complete author and subject index. The following chapters will be of service to those whose activities include the production, processing and appraisal of the nutritional value of milk: Proteolytic Enzymes; Nonproteolytic Enzymes; Fat Metabolism; The Metabolism of Proteins and Amino Acids; The Water Soluble Vitamins; Fat-Soluble Vitamins; Nutrition; Relation of Soil and Plant Deficiencies and of Toxic Constituents in Soils to Animal Nutrition, and Bacterial Metabolism. Other subjects which may be of interest to dairy industry workers are: biological oxidations and reductions, chemistry of the carbohydrates, glycosides, compounds of sulfur, carbohydrate metabolism, biochemistry of nucleic acids, purines, pyrimidines, creatine, creatinine, and hormones.

K.G.W.

574. **Indian Indigenous Milk Products.** W. L. DAVIES. Published by Thacker, Spink and Co., Ltd., Calcutta, India. 1940. 96 pp., heavy paper cover, Rs. 1/8 (about 40-45 cents.)

This small but interesting text of the dairy products of India was written by W. L. Davies, Director of Dairy Research, Government of India, Calcutta, and author of the book "The Chemistry of Milk." This text is divided into seven chapters on: Composition and Behavior of Milk; Indian Milk and Whole Milk Products—Khoa and Rabbri; Fermented Milk Products, Dahi and Lassi; Desi Butter, Ghee; Miscellaneous Products, Creams, Cheese, Channa; Utilization of Indian Milk for Manufacture of Western (World) Products.

One of the appreciated advantages of the volume is the comparison made between the various Indian milk products and comparable products of greater familiarity to us. In addition, the manufacture of the representative Indian products is given in sufficient detail, and technical control terms that the processes can be readily visualized and probably duplicated. For example, times and temperatures, titratable acidity, pH, color, physical appearance, keeping quality, native utensils and so forth are included to describe the making of the various products. Malai and sar are classified as clotted cream, khoa and rabbri as dried, evaporated and sweetened condensed (khoa and rabbri 6-1, Kheer 3.5-1). Channa is comparable to soft cheese, dahi and lassi resemble the fermented buttermilk, desi butter (soured

cream "country butter"), and ghee, heated melted butter. An interesting discussion is included on the probabilities and problems of making "western products" from the native milks. The discussion on ghee, an important dairy food item, is quite complete. The text will be of value to those seeking to develop new products and new uses of products.

K.G.W.

BACTERIOLOGY

575. **Single Colony Isolation of Anaerobes.** L. GREENBURG, Dept. of Pen-sions and National Health, Ottawa, Canada. *Canad. Pub. Health Jour.*, 32: 84-85. 1941.

A method of isolating anaerobes which requires no special apparatus is described. A very light inoculum is transferred to 5 ml. of sterile broth and mixed. A loopful of this broth is then transferred to the special semi-solid media which has been heated in boiling water to displace oxygen and then cooled at 45° C. For most *Clostridium* species studied, incubation was at 22° C. overnight followed by holding at 37° C. Cultures are removed from the incubator when colonies are of a suitable size. The medium used is: Proteose peptone, 10 grams; Tryptone, 10 grams; Sodium Thioglycollate, 1 gram; Agar, 3 grams; Distilled water, 1,000 ml.

This medium is adjusted to pH 7.4, dispensed into tubes and autoclaved at 15 pounds pressure for 20 minutes.

O.R.I.

576. **Laboratory Procedures in Staphylococcal Food Poisoning.** R. J. WILSON, Univ. of Toronto. *Canad. Pub. Health Jour.*, 31: 607-612. 1940.

A method is outlined whereby individual strains of staphylococci may be identified by their toxigenic properties. The regular methods of classifying these organisms by means of carbohydrate utilization, chromogenicity, etc., are unsatisfactory to incriminate enterotoxin producing strains. Identification is made by tests for hemolysis, color and the kitten test, in addition to other procedures.

O.R.I.

577. **A Study of Methods for the Detection of the Presence of Coliform Organisms, in Water.** N. J. HOWARD, A. G. LOCKHEAD AND M. H. MCCRADY. *Canad. Pub. Health Jour.*, 32: 29-36. 1941.

Results are presented from five laboratories where the brilliant green bile test was compared with the A.P.H.A. "completed test" as confirmatory procedures for the detection of coliform organisms in water supplies after lactose broth had shown positive results in the presumptive test. The results indicate that the brilliant green bile method is quite as satisfactory as the "completed test."

O.R.I.

BREEDING

578. **Estimates of Producing Ability in Dairy Cattle.** G. E. DICKERSON.
Jour. Agr. Res. 61, No. 8: 561. October 15, 1940.

Lifetime butterfat production records of 274 Holsteins from 41 herds were studied to determine what adjustments for environmental influences are advisable and the relative usefulness of five kinds of adjusted records (240-day, 305-day, 365-day, total lactation, and testing year) in selecting cows for producing ability. The average within-herd correlation between records of the same cow (repeatability) was the criterion used in evaluating adjustments and comparing kinds of records.

Age-correction significantly increased the repeatability of all five kinds of records. Correction for calving interval to a 365-day basis increased the repeatability. Season of calving was a relatively unimportant source of variation in production and no correction factors were given. It was concluded that the age-corrected 305-day record was probably the most satisfactory for selection purposes because of its early availability, ease of computing and high degree of correlation with the average lifetime record.

W.J.C.

579. **Judging Dairy Cattle on the Basis of Type and Records of Production.** W. W. SWETT AND R. R. GRAVES, Bureau of Dairying, Washington, D. C. U.S.D.A. Miscellaneous Publication 409. 29 pp. Jan., 1941.

In order to place cows on a combined type and production basis a system for numerical evaluation is presented. Ten per cent of the yearly butterfat production records corrected to maturity are used as a base. If equal emphasis is placed on type and production the extreme scores for type are the same as the extreme values of the 10 per cent of the butterfat production. As an example, if 10 per cent of the highest and lowest producing cows is 98.8 and 40.2 respectively, then the best type cow is scored 98.8 and the poorest 40.2 regardless of the range of differences in type. Intermediate types may be credited with scores of adjusted intervals, depending upon variations of the type in the group. The final placing is based upon a summation of the type "score" and the production "score."

A more complicated system of scoring proven bulls involves: (a) rating on average type of daughters, (b) average production of daughters, (c) average increase of butterfat production by daughters, and (d) per cent of daughters that increased production. The scores for type and average increase in butterfat production for the highest and lowest sires have the same numerical values, respectively, as the highest and lowest average butterfat production of the daughters. The values of all four considerations are summated for final placing.

W.E.P.

580. Early Recognition of the Freemartin Condition in Heifers Twinborn with Bulls. W. W. SWETT, C. A. MATTHEWS AND R. R. GRAVES. Jour. Agr. Res., 61, No. 8: 587. Oct. 15, 1940.

It is estimated that about 11 out of 12 heifers twinborn with male calves will be freemartins. In a study of 17 heifers twinborn with male calves all those that were kept to breeding age proved to be sexually abnormal and incapable of reproduction. Two of the 17 heifers were found on post mortem to have normal genital development.

Characteristics found to be associated with freemartins were: 1. highly retarded udder development or atypical mammary gland development; 2. enlarged clitoris; 3. the presence of a fold of skin often containing a cord which extended along the median plane of the body, part or all the way from a point above the rear attachment of the udder to the navel. One to all three of the characteristics were found in the 15 freemartins studied.

W.J.C.

BUTTER

581. Leaky-bodied Butter. S. T. COULTER, Univ. of Minnesota, St. Paul. Natl. Butter and Cheese Jour., 32, No. 6: 14. 1941.

Leakiness in butter is measured by determining its loss of weight when 1 pound prints are subjected to high vacuum for 10 minutes followed by removal of free moisture in an air blast. Leakiness is decreased by the use of fat found in Summer butter; by thorough cooling of cream; by low temperatures of working; probably by adding water for standardizing at the start of working; by salting while the butter is in the granular form; by thorough working; and by avoiding the addition of too much water in standardizing.

W.V.P.

CHEESE

582. Au Sujet de L'adaptation des Butyromètres a Lait pour le Dosage de la Matière Grasse dans les Fromages. (On the Subject of the Adaption of Milk Butyrometers to the Determination of Fat in Cheese.) MADAME JEAN BOURGEOIS. Le Lait, 20: 403-407. 1940.

Confusion exists as to the correct factor to use in calculating the fat content of cheese when 2.5 g. of sample are used in the milk butyrometer. One factor was suggested on the basis that the milk butyrometer was calibrated on the basis of volume while the second factor is based on weight. Literature is reviewed and it is suggested that the factor, 4.4 based on weight calibration, is more reliable.

O.R.I.

583. Preliminary Observations on the Survival of *S. typhi* in Canadian Cheddar-type Cheese. L. E. RANTA AND C. E. DOLMAN, Univ. of Toronto. Canad. Pub. Health Jour. 32: 73-74. 1941.

Five ml. amounts of a standardized *S. typhi* broth culture were intimately mixed with 30 cc. of minced British Columbia Cheddar cheese and stored in sealed Petri dishes at 68° F. At intervals, samples were withdrawn, macerated in sterile saline and single drops spread on plates of selective media. In three trials, survival occurred until twenty-sixth, twenty-eighth and twenty-sixth day respectively. In the refrigerator, samples were positive for 17 weeks after inoculation.

In the second series of trials a standardized suspension of *S. typhi* was made in Seitz-filtered whey. This was poured over unmacerated cheese in a Petri dish. Survival time was approximately the same as in the above trials. In a third trial, it was shown that *S. typhi* possessed the ability to penetrate cheese for 4-5 cm.

The authors suggest that these observations point out the need for significant changes in control measures in the manufacture and marketing of Canadian Cheddar cheese.

O.R.I.

584. Factors Affecting the Survival of *Streptococcus Pyogenes* in Cheese. M. W. YALE AND J. C. MARQUARDT. Jour. Milk Tech., 3: 326-333. 1940. (Also published in the 14th Annual Report of the N. Y. State Assoc. of Dairy and Milk Insp., 1940.)

In cheese made from milk inoculated with *S. pyogenes*, the variety of the cheese, its moisture content, and curing temperature were some of the important factors affecting the length of survival of these organisms in the cheese.

In cottage cheese where the minimum pH values were about 4.5 the organism was not recovered at the end of 24 hours.

When pasteurized milk inoculated with *S. pyogenes* was added to 28 hour old curd, no *S. pyogenes* were recovered 20 hours later.

S. pyogenes survived for 28 to 51 days in limburger cheese containing 42.8 per cent moisture and for only 9 to 14 days in another lot containing 49.3 per cent moisture. In the case of cheddar cheese the organisms survived much longer, curing temperature being a factor. In cheese cured at 45° F. the organisms survived for over 18 weeks, while in duplicate cheese cured at 62° F., they survived for only between 9 and 11 weeks. At 50° F. they survived for less than 18 weeks.

L.H.B.

585. Pasteurization for Cheesemaking. E. C. DAMROW, Fond du Lac, Wis. Natl. Butter and Cheese Jour., 32: No. 6: 10. 1941.

The regenerative system of pasteurization is most practical because it uses steam and water efficiently. Such a system with a capacity of 6500 pounds of milk per hour, uses seven and one-half horse power per hour, while water requirements range from nothing up to 2500 pounds depending on the temperature of incoming milk.

W.V.P.

CHEMISTRY

586. *La Fabrication D'acide Lactique Pur.* (The Manufacture of Pure Lactic Acid.) G. GENIN. *Le Lait*, 20: 412-417. 1940.

The manufacture of crude and purified lactic acid is reviewed and industrial standards of purity are outlined. Six different methods of purifying are described including (1) purification by crystallization as calcium lactate; (2) or as zinc lactate; (3) extraction from aqueous solution using such solvents as isopropyl ether; (4) oxidation of the organic impurities by chromates, permanganates, ozone, etc.; (5) fractional distillation, and (6) separation and hydrolysis of lactic esters. The latter is the method of Smith and Claborn (*Indust. Engin. Chem. News Ed.*, 7: 641. 1939). O.R.I.

CONCENTRATED AND DRY MILK;
BY-PRODUCTS

587. *Analyse des Laits Altérés ou Coagulés.* (The Analysis of Altered or Coagulated Milk.) E. G. VOIRET, Municipal Lab., Lyon. *Le Lait*, 20: 407-410. 1940.

A laboratory-sized, motor-driven mill is described and illustrated which has proven satisfactory for the reconstituting of milk samples which have developed abnormal consistencies. The motor is mounted vertically and operates several stirrers in the base of the hopper. In addition, a disc, also integral with the motor shaft and stirrers, helps emulsify the samples. O.R.I.

588. *Deux Nouveaux Défauts du Lait Concentré Sucré.* (Two New Defects of Sweetened Condensed Milk.) C. A. CECILIA, Vet. School, Madrid. *Le Lait*, 20: 385-390. 1940.

Two flavor defects of bacterial origin in sweetened condensed milk are described. One possesses the odor of fish while the second has the odor and taste of glue. In the case of the fishy flavor samples, the cans were greatly swelled, the contents coagulated and stuck to the sides and yellow-brown in color. The titratable acidity was 0.5-0.7 per cent and the Breed count 20,000-50,000 per gram on the samples which had been previously unopened. *B. mycoides* and *B. mesentericus* were isolated either singly or in combination in these samples. In no case was *Proteus ichthyosimus* Hammer found. While it was found impossible to reproduce this defect by inoculating sweetened condensed milk with these organisms and with *B. pseudo anthracis*, alone or in combination, nevertheless, it is still felt by the author that their presence contributes to this off flavor.

Cans containing milk possessing the glue flavor were normal in external appearance although some swelled after being incubated for 48 hours. The

color, viscosity, and solubility were normal. Acidities ranged from 0.3 per cent to 0.8 per cent. Plate counts ranged from 1,000 to 15,000 colonies per gram. Some of the small colonies isolated from the plates produced a glue flavor grown on lactose agar. The following characteristics were found for the organism: A *Strepto bacillus* producing longer chains in milk than in other media; well capsulated especially if allowed to stand in the incubator for a few days; cells display metachromatic granulations; dimensions are less than those of *B. anthracis*; non-motile and does not form spores.

It may be considered as a facultative aerobe growing up to 55° C. Milk is coagulated in 72 hours. The name *Thermobacterium mathiacolle* has been proposed for this organism. O.R.I.

DISEASE

589. Simplified Cultural Methods for the Diagnosis of Streptococcic Mastitis. RALPH B. LITTLE, Rockefeller Inst., Princeton, N. J. Internatl. Assoc. Milk Dealers, Assoc. Bul., 33rd yr., 21: 565-570. Apr. 1941.

The Hotis test as originally reported by Hotis and Miller was modified by the addition of sodium azide to brom cresol purple which prevented the development of coliform organisms without hindering the growth to *Str. agalactiae*. For a more critical test Edwards selective liquid medium was used. Serological identification of streptococci can be made from Edwards selective medium growth. The above tests result in a saving of time and simplification of equipment. E.F.G.

590. A New Group of Sterilizing Agents for the Food Industries and a Treatment for Chronic Mastitis. F. M. SCALES AND MURIEL KEMP, Sheffield Farms Co., New York City. Internatl. Assoc. Milk Dealers, Assoc. Bul., 33rd yr., 19: 491-519. Apr. 1941.

The literature with reference to wetting agents as germicides is reviewed and the results of new work reported.

The wetting agents have the advantage over chlorine solutions in their wetting properties causing uniform films on surfaces, and also in stability and no corrosive action. Death of the bacterial cell is brought about by the wetting quality permitting penetration of the mildly toxic agent to the protoplasm of the bacterial cell. The wetting agents used included Aerosol OT, Naccenol, Modinal E S, Intramine Y, Triton 720, Aerosol D G A, Turkey Red Oil, Mellol No. 100.

The wetting properties of many of these preparations as sold is reduced and their germicidal properties are reduced by admixtures of neutral salts as sodium sulphate or sodium chloride. Aerosol O T in pure solution gave killing effect in 3 minutes at room temperature on high concentrations of *Staph. aureus* in solution of pH 4.0 acidified with phosphoric acid. Ther-

moduric organisms may be killed in plant work by wetting agents in a 0.03 per cent concentration at pH 4.0 at a solution temperature of 22° C. and up. For farm utensils the following procedure is suggested. Wash the utensil in an alkaline solution plus the wetting agent which will considerably reduce the number of organisms. Rinse with warm water and finally give a second treatment with a solution of cleaning powder and wetting agent which will sterilize in ten minutes at 43.5° C. and up. The cleaning powder used should contain sodium tetra phosphate to remove any lime deposits from the utensils. Spores of *B. subtilis* are not acted upon readily even by the best of the wetting agents Aerosol O T. Zephiran is a good agent to use for cows udders since it is effective in neutral solutions. For udders a 1 to 5000 dilution is recommended. This compound has the ability to reduce the surface tension of water to approximately half, resulting in suds upon shaking. It is suggested that injection of wetting agents directly into the milk cistern may hold possibilities for treatment of chronic mastitis. Corrosion by the wetting agent acidified with phosphoric acid (pH 4.0) was approximately one sixteenth as extensive on tinned copper as hypochlorite solutions of 100 p.p.m. available chlorine. Sterilizing solutions of wetting agents will cost from $\frac{1}{3}$ as much to the same as chlorine. E.F.G.

591. **Contagious Abortion of Cattle and Undulant Fever in Man.** J. S. FULTON, Univ. of Saskatchewan. *Canad. Pub. Health Jour.*, 32: 194-198. 1941.

While contagious abortion has been known to be common in Saskatchewan for many years, it is only recently that a blood testing service has revealed the extent of human Brucellosis. Since 1933, the percentage of reacting bovine blood samples has been halved as have also the percentage of positive human blood samples.

Results are given of a study of the milk of 60 reacting cows in which 70 per cent were found to yield *Br. abortus* in their milk. The organism survived at least 18 months in sterile milk at icebox temperatures.

Details are given of the Saskatchewan plan of control whereby municipalities are encouraged to foster eradication programs. O.R.I.

592. **The Present Status of Milk-Borne Disease Hazards.** C. E. DOLMAN, Univ. of British Columbia. *Canad. Pub. Health Jour.*, 32: 183-193. 1941.

National public health statistics usually indicate a far lower incidence of disease than actually occurs. It is suggested that milk-borne diseases may result in 1500 cases and 130 deaths in Canada per year as a result of typhoid, paratyphoid and scarlet fevers, and septic sore throat. Cases of tuberculosis and Brucellosis of bovine origin are difficult to blame on milk since they do not occur in epidemic form.

The rate of incidence of such diseases as tuberculosis, Bang's disease and streptococcal and staphylococcal mastitis is estimated and the relation of these bovine diseases to human diseases pointed out by means of references to recent literature.

The need for control of typhoid and other types of carriers is discussed, the former particularly in the case of cheese producers. The use of pasteurization in the preparation of all dairy products is particularly stressed as a preventive measure.

O.R.I.

593. **Transmission of Animal Disease to Man through Milk.** MAZYCK P. RAVENEL, Univ. of Missouri. *Canad. Pub. Health Jour.*, 32: 174-182. 1941.

Although this treatise is not extensive nor complete, it is a very clear discussion of three animal diseases transmissible to man through milk. The diseases dealt with are: Brucellosis, the pathogenic cocci, and tuberculosis. Enough of the pioneer work is reviewed to make the account very interesting.

O.R.I.

594. **The Significance of the "Ceased" Reactor to Bang's Disease.** B. A. BEACH, M. R. IRWIN, AND L. C. FERGUSON. *Jour. Agr. Res.*, 61, No. 1: 75. July 1, 1941.

Twenty-one "ceased" reactors (those animals that have lost their agglutinin titer to *Brucella abortus* following infection) were allowed to come in with 54 animals from Bang-negative herds through either 1 or 2 gestation periods for each of the normal cows. Except for a transitory udder infection in one "ceased" reactor and a low titer in the blood serum of one normal cow (no evidence of the organism) no evidence of infection was obtained by culture or guinea pig injection at the time of calving. The results of the experiment indicated that it is relatively safe to allow "ceased" reactors to contact normal or non-infected cows.

W.J.C.

595. **Ineffectiveness of Proprietary Remedies and Other Drugs in the Control of Bang's Disease with Special Reference to "3-V Tonic" and "Bowman's."** A. B. CRAWFORD AND B. A. BEACH. *Jour. Agr. Res.*, 60, No. 8: 565. Apr. 1, 1941.

The history of drugs or other therapeutic chemicals in the treatment of Bang's disease has been negative so far as finding any substance which has a specific action on *Brucella* organisms in the tissues of animals. In spite of this fact so-called remedies for Bang's disease still appear on the market. Two of these alleged remedies "3-V Tonic" and "Bowman's" were tested for their effectiveness.

In testing "3-V Tonic" a group of 19 pregnant heifers negative to the agglutination test was fed this product prior to exposure to virulent strains

of *Brucella abortus*; a second group of 20 heifers was fed the preparation prior to and subsequent to exposure; and a third group of 19 heifers, as controls, received only exposure. *Brucella abortus* organisms were found in the colostrum or uterine material of all cows following parturition. All cows either aborted or gave birth to weak calves.

In the testing of Bowman's product a group of 19 pregnant heifers was fed the preparation prior and subsequent to exposure to *Brucella abortus* and a second group of 20 heifers as control were only exposed. All heifers in both groups except one in each group either aborted or gave birth to weak calves and *Brucellus* organisms were recovered in uterine material or colostrum of all 30 animals. Feeding the material for 8 months after infecting did not decrease the blood titer.

It was concluded that the "3-V Tonic" and Bowman's were ineffective in preventing or curing Bang's disease. W.J.C.

596. Treatment of Nephrosis with Vitamin A and Unsaturated Fatty Acid Therapy. GEORGE W. CALDWELL. Arch. Ped., 57, No. 4: 247. 1941.

A case is reported where a seven-year-old girl suffering from nephrosis was treated by feeding 40,000 to 100,000 units of vitamin A per day and 2 to 6 teaspoonfuls of corn oil (unsaturated oil) daily. The case responded quite favorably to this treatment which suggests that nephrosis is a deficiency disease in addition to any infectious factor. W.J.C.

597. Suprarenal Gland and Lactose in the Treatment of Major Disorders in Childhood. STEPHEN D. LOCKEY. Arch. Ped., 57, No. 11: 725. 1940.

Whole suprarenal gland concentrate plus lactose (1 to 111 drams daily depending on age) were fed by mouth to 163 cases of major allergic disorders of children (eczema, asthma, hay fever) with a clinical improvement in approximately 82 per cent of the cases. In bronchial asthma the suprarenal gland concentrate and lactose reduced the frequency of attack and the attacks were milder when they did occur as compared to the control groups. Suprarenal gland concentrate and lactose improved the general health condition of the children as compared to the controls. The administration of the suprarenal gland concentrate and lactose to children afflicted with eczema showed very marked improvement in 3 to 5 days. W.J.C.

598. The Leucocyte Count and the Chloride Content of Milk from Bovine Udders with Mild Streptococci Infections. J. FRANK CONE. J. Milk Tech., 3: 341-345. 1940. (Also published in the 14th Annual Report of the N. Y. State Assoc. of Dairy and Milk Insp., 1940.)

The chloride content and leucocyte count of milk failed to reliably distinguish between mildly infected quarters and non-infected quarters. In conjunction with bacteriological tests the chloride content and leucocyte count of samples from the various quarters of the same udder gave valuable information in support of the cultural method.

An abrupt rise in the chloride content and leucocyte count of the milk from cows tested periodically strongly indicates the beginning of infection, even though these values may not exceed the values arbitrarily set as indicating mastitis.

The leucocyte count is a more reliable index for detecting mastitis than is the chloride content.

L.H.B.

FEEDS AND FEEDING

599. Untersuchungen über die Veränderung der Nährstoffe durch künstliche Trocknung. (Studies Relating to Feed Drying Techniques and Nutritive Value Changes). W. LENKEIT AND M. BECKER. Ztschr. f. Tierernährung und Futtermittelkunde, 4: 20-37. 1940.

This article deals with procedures to preserve carotene in beet leaves and red clover. Sixty to 90 per cent is retained by shredding and pressing. In the normal hay drying procedures only 30 per cent of the carotene content is retained.

J.C.M.

600. The Feeding Value and Nutritive Properties of Citrus By-products. II. Dried Grapefruit Pulp for Milk Production. P. T. DIX ARNOLD, R. B. BECKER, AND W. M. NEAL. Florida Agr. Exp. Sta. Bul. 354. 14 pp. 1941.

In feeding trials where it composed 40 per cent of the T.D.N. of the ration, dried grapefruit pulp was found to have about the same value in the dairy ration as dried beet pulp. It is palatable to dairy cows and produced no detectable flavors in the milk. Twenty-day digestion trials on 4 steers showed 24.8 per cent of the crude protein, 71.5 per cent of the crude fiber, 92 per cent of the nitrogen-free extract and 79.4 per cent of the crude fat to be digested. The dried product was calculated to contain 1.2 per cent digestible crude protein and 76.0 per cent T.D.N.

W.E.P.

601. Estimating the Quantity of Settled Corn Silage in a Silo. J. B. SHEPHERD AND T. E. WOODWARD. U.S.D.A. Cir. 603. 11 pp. April, 1941.

Data and table are presented giving average weight and dry matter per cubic foot for silage at varying depths for corn averaging 27.63 per cent dry matter. Except for the first three feet (which were not as heavy) the table shows higher weights per cubic foot than the table of Eckles, Reed

and Fitch. The weights per cubic foot increased rapidly for the first five feet and more slowly up to depths of 30 feet, below which the weights were practically constant. Settling continues for 30 days.

Stage of maturity affected silage weight. If the corn is well eared but immature, add 5 per cent to weights; if only a fair number of ears, deduct 5 per cent and if it has few or no ears deduct 10 per cent. If the corn contains 31 to 33 per cent dry matter and ears are fully dented, deduct 5 to 10 per cent. If corn contains 34 to 36 per cent dry matter and ears are fully dented, deduct 15 to 20 per cent.

Finely cut silage weighs more. When cut in $\frac{5}{8}$ to $\frac{3}{4}$ inch lengths, deduct 5 per cent as compared to $\frac{1}{2}$ inch lengths. W.E.P.

FOOD VALUE OF DAIRY PRODUCTS

602. **The Pigments, Vitamins and Enzymes of Milk in Relation to Changes in Flavor and Nutritive Value.** DAVID B. HAND AND PAUL F. SHARP, Cornell University, Ithaca, N. Y. Internatl. Assoc. Milk Dealers, Assoc. Bul., 33rd yr., 17: 460-463. March 1941.

It is pointed out that the oxidation changes which take place in pasteurized milk and other dairy products resulting in flavor changes and loss of nutritive value are only partially understood.

Vitamin C behaves as an antioxidant and the addition of relatively large amounts of it to milk will retard the development of oxidized flavor. Experiments have shown that riboflavin, the fluorescent green coloring matter in whey, is responsible for the oxidation of vitamin C in light. Riboflavin when combined with a specific protein becomes a catalyst or enzyme. About 5 per cent of the total riboflavin of cows' milk is so combined. The flavoprotein or Shardingner enzyme is concentrated on the surface of the fat globule. If cream rich in riboflavin is wanted it should be separated raw since pasteurization breaks down the flavoprotein and there is then no concentrating effect from separation. Carotene is considered to be an antioxidant. Tallowness may develop as the carotene in cream is bleached. Oxidized fats exert a destructive effect upon both vitamin A and E. The interaction of vitamin C and hydrogen peroxide offers a clue to the way vitamin C retards the oxidation of fats. The initial stage in the oxidation of fat is thought to be the formation of an organic peroxide. It is possible that vitamin C reduces this peroxide as fast as it forms. E.F.G.

603. **Some Observations on Canadian Nutrition.** E. W. MCHENRY, Univ. of Toronto. Canad. Pub. Health Jour., 31: 584-588. 1940.

Some fairly accurate information has recently been accumulated regarding deficiencies in Canadian dietary habits among two income groups. The

average values for 100 families having incomes below \$1,000 per year showed that the supply of calories was 76.5 per cent of the recommended standard; protein supply, 77 per cent; calcium, 69 per cent; and iron, 62 per cent. In the the income group between \$1,500 and \$2,400 these values were: total calories, 93 per cent of standard; protein, 95 per cent; calcium, 116 per cent; and iron, 99 per cent.

While this evidence suggests that undernutrition is due to financial inability to buy proper food, other factors were found to contribute to this picture particularly lack of nutritional knowledge. For low income groups, the use of more milk, skim milk, and cheese is advocated as well as the use of breads containing whole wheat flour or wheat germ to increase the intake of the B group of vitamins. O.R.I.

604. **An Educational Program to Raise Nutrition Levels through Increased Milk Consumption.** M. FRANCES HUCKS, Milk Foundation of Toronto. *Canad. Pub. Health Jour.*, 32: 158-162. 1941.

A co-operative, producer-distributor, non-commercial enterprise which is now in its fourth year is known as the Milk Foundation of Toronto. This foundation has had the support of other health groups and its program has been readily accepted, particularly by school authorities. Its principal aim is to foster interest in, and promote greater consumption of milk. In addition to its school program, newspaper advertisements, lectures, food demonstrations and a technicolor sound film have been widely used. O.R.I.

605. **Vitamin C—Practical Considerations.** I. A. GOULD. Michigan State College, East Lansing, Mich. *Milk Dealer*, 30, No. 6: 108-109. March 1941.

A brief discussion is given of vitamin C, with a review of the factors influencing ascorbic acid in milk. C.J.B.

606. **Effects of Milk Diets on Guinea Pigs.** ROSALIND WULZEN AND ALICE BAHRS, Oregon State College, Corvallis, and St. Helen's Junior College, Portland, Ore. *Am. Physiol. Soc. Proc.*, p. 312. Apr., 1941.

Groups of young guinea pigs were fed rations of whole raw milk, pasteurized whole milk, raw skim milk and pasteurized skim milk. Animals fed raw whole milk grew excellently and at autopsy showed no abnormality of any kind. Those on the pasteurized milk rations did not grow as well and developed a definite syndrome, the first sign of which was wrist stiffness. On pasteurized skim milk ration the syndrome increased in severity until the animals finally died in periods ranging from a month to a year or more. They showed great emaciation and weakness before death but remained in normal posture and had no tendency to paralysis of the limbs. Upon

autopsy the muscles were found to be extremely atrophied and in most cases were streaked with closely packed, fine white lines of calcification running parallel to the muscle fibers. There were often lumps of tricalcium phosphate deposited under the skin, in the joint regions, between the ribs and indiscriminately in many body organs including heart and aorta.

It was found that raw cream given by mouth had power to cure the original wrist stiffness. An extract was made from raw cream which was able in a few days to restore the stiff wrists of affected animals to their normal limber condition. This active substance was found by Romeo Gouley to be methylvinylketone and was successfully synthesized by him. The synthetic product had active curative power.

When cod liver oil, one-half per cent, was substituted for carotene in the skim milk ration, in addition to stiffness the animals quickly developed paralysis. Their hind legs dragged and locomotion soon became impossible. It was found that synthetic methylvinylketone was able to restore locomotion to those animals provided they were not moribund.

D.L.E.

HERD MANAGEMENT

607. **Bull Quarters, Breeding Chute, Yard-house.** J. G. HAYS, A. J. BELL AND C. H. JEFFERSON. Mich. State College Ext. Bul. 32. 12 pp. March 1941.

Details with illustrations are given for building the bull house, yard and breeding chute.

W.E.P.

ICE CREAM

608. **Pasteurizing Ice Cream Mix.** J. M. BRANNON, Univ. of Illinois. Ice Cream Field, 37, No. 5: 26. 1941.

The evidence available leaves little doubt that pasteurization at 150° F. to 160° F. will destroy all disease germs in ice cream mixes. It is the author's opinion that high bacterial content in ice cream may be due either to (1) lack of vigilance in selecting ingredients or (2) lack of thorough cleaning of equipment.

Mention is made of an earlier survey which showed that only 17 per cent of the ice cream sold in Illinois had bacteria contents of 100,000 per gram or less. It is also pointed out that increase in the "plate count" of ice cream during freezing is probably due largely to the breaking up of bacterial clumps.

The author expresses the view that ice cream mix is generally sufficiently pasteurized and the largest number of bacteria in commercial ice cream are picked up from the equipment used after pasteurization.

W.C.C.

609. **Refrigeration Troubles.** P. B. REED, Servel Inc., Evansville, Ind. Ice Cream Field 37, No. 4, 21. 1941.

From the point of view of preventing difficulties in new installations three principle sources of trouble are discussed.

(1) *Leaks*. The author subscribes to the view that "all refrigeration systems leak, but some leak faster than others." The possibility of leakage in the highsides or lowsides obtained from reputable manufacturers is rather remote at present. Furthermore the use of soldered connections to replace flare nuts and flanges is a step in the right direction. Some leaks are too small to be easily detected, hence it is recommended that an excess of 10 to 25 per cent be carried in a highside receiver as a practical reserve.

(2) *Lubrication*. The early failure of mechanical parts of a refrigeration compressor is almost always due to imperfect lubrication. It is stated further that dilution of oil or washing by liquid refrigerant can be minimized by careful adjustment of expansion valves, proper location of thermostatic expansion valve bulbs and the selection of expansion valves.

(3) *Foreign matter in the systems*. Air and other "non condensable" gases are common offenders. They raise the head pressure which reduces the capacity and increases the power consumption and at the same time increase the hazard of chemical action in combination with the refrigerant. It is recommended that a high grade vacuum pump be used in addition to the old practice of "purging."

Moisture in the system also may be a source of considerable trouble. Several precautions are indicated as to possible sources of moisture. Dehydrators are sometimes used to advantage but it is claimed that "anti-freezes" are not substitutes for the elimination of moisture in refrigeration systems.

W.C.C.

610. The Profitable Production of Novelties. H. J. BROWN, Central Ice Cream Co., Chicago. Ice Cream Field, 37, No. 5: 14. 1941.

Emphasis is made of the necessity of selecting the novelties that will be acceptable to the consuming public, also the fact that relatively large scale production is required because of the special equipment necessary to manufacture such novelties. It is stated that "stick novelties" such as chocolate coated bars and frozen water ices usually account for the largest volume, and that these novelties require the greatest investment in equipment and manufacturing space.

It is stated that proper plant layout, low brine temperature, elimination of waste at filling molds, careful defrosting of filled molds, and use of suitable automatic machinery all contribute towards plant efficiency. Several operations can now be made automatic, but it is claimed that each type of operation has its own particular problems which must be considered as a basis of eliminating waste.

The selection of raw material is of most importance in profitable novelty operation but the author states that in order to gain sales volume novelties must be of high quality.

W.C.C.

611. Soda Fountain Trends. R. H. CRANE, Liquid Carbonic Corp. Ice Cream Field, 37, No. 5: 10. 1941.

The author states that the soda fountain of today is the result of a slow evolution over the past thirty-five years; further, that future improvements in the fountain itself can be expected to be slow. It is claimed, however, that the carbonated beverage business is undergoing the greatest growth of almost any industry and along with the increased consumption of these products people are buying ice cream, malted milks and other fountain products.

There is a trend towards the installation of larger soda fountain units with more attention being given to sanitation and pleasant environment associated with "modernization." Cleanliness and food health are of major importance to the consuming public and certain progressive fountain operators are capitalizing on this fact, the author states.

The reverse wall fountain accounts for 65 per cent of fountain layouts longer than 18 feet and the view is expressed that this type installation will become standard. It is also reported that a fountain may become identified in the minds of the public as a restaurant if it encroaches too far into the food business, and because of this there is a tendency towards limiting the menu in the fountain where its service operations are open to the view of the public. Other trends mentioned are: wider top slabs at the fountain, more knee room for the customers, added color and utility through use of plastics.

W.C.C.

612. New Uses for Dry Ice. ANONYMOUS. Ice Cream Field, 37, No. 4: 10. 1941.

It is stated that the annual production curve for dry ice shows a high peak from May 15 to September 15 and that any development tending to equalize the production would at the same time tend to lower production and distribution costs. Added usage in the industrial field should likewise lower costs.

Regarding more efficient use of dry ice it is claimed that (1) For delivery truck refrigeration proper insulation and the use of icefin plates offer the maximum efficiency. The conduction plate which controls temperature is the secret of this system. (2) The Siphotherm dry ice cabinet, because it requires less frequent icings and because it uses less dry ice seems best adapted to permanent operation. (3) The adoption of paper cans for ice cream makes the use of corrugated cartons and dry ice obligatory, but there is an efficient compromise between cost of carton and the amount of dry ice necessary for refrigeration purposes. Paper cartons made of "triple corrugated" paper box board add to convenience in assembling boxes of a given efficiency.

Descriptions are given of several types of paper cartons which are now being used by the ice cream industry. W.C.C.

613. Variegated Ice Cream. J. J. SHEURING, Univ. of Illinois. Ice Cream Field 37, No. 4: 18. 1941.

Variegated ice cream is prepared by injecting flavored syrups or gels into ice cream as it comes from the freezer, in such a way as to give a wavy ribbon-like appearance throughout the finished product. It is stated that the flavoring should be injected into the ice cream as near the outlet of the package-filling attachment as possible in order to prevent the flavoring from being "smeared" with ice cream. Improvised methods are mentioned for continuous as well as batch freezers.

Settling of the flavoring material is obviously objectionable. The following methods of preventing settling are given: (1) The ice cream should be sufficiently firm or stiff when it leaves the freezer. (2) The flavoring material should be cold (at least 40° F. and preferably 32° F.) and be high in viscosity although fluid enough to be pumped satisfactorily. (3) The ice cream should be hardened as rapidly as possible and distributing cabinets should be maintained at a relatively low temperature. It is also pointed out that iciness may be caused if the flavoring material added is above 40° F., if the sugar content of the flavoring is too low, if heat shocking occurs or if the flavoring is not properly stabilized.

Many flavoring materials are now available for this type of ice cream. Chocolate, raspberry and strawberry are the most common. It is stated that fruits used for this purpose may be either fresh, frozen packed or canned, but in any case they should be in the form of puree or very finely ground. Marshmallow syrup, butter scotch fudge and caramel fudge are mentioned as other popular flavors. W.C.C.

614. Variegated Ice Cream. C. D. DAHLE, Pennsylvania State College, State College, Pa. Ice Cream Field, 37, No. 4: 14. 1941.

Variegated ice cream, which started with chocolate ribboned through vanilla ice cream, has been sold under a variety of names. It now includes many fruit flavors, although it is stated that chocolate, raspberry and strawberry are the most important flavors.

Many types of pumps and fillers are available for satisfactory use with the continuous freezers, and some of the equipment now in use with the batch freezers, although not sanitary, result in fairly satisfactory appearing finished products according to the author. Certain very small manufacturers have been able to make this type of ice cream by pouring the flavor syrup into the ice cream as it is drawn from the freezer.

The following essentials are given by the author for desirable fruit flavors for variegated ice cream: (1) proper color, (2) correct amount of

stabilizer (usually pectin) to give desirable body, (3) proper sugar content, and (4) satisfactory acidity.

The main steps in the preparation of such fruit flavors are outlined as follows: grind or pulp the fruit, calculate the sugar content of the fruit and increase it to about 40 per cent, adjust the final acidity and the pectin content to 1 per cent or more.

W.C.C.

615. **Dramatizing the Soda Fountain.** W. C. SHOEMAKER, Read Drug and Chemical Co., Baltimore, Md. Ice Cream Field, 37, No. 4: 36. 1941.

Emphasis is placed on the necessity of using suitable displays to dramatize the soda fountain. Products sold at the soda fountain get proportionately less newspaper, magazine and radio advertising than drugs and patent medicines, toilet articles or tobacco and cigarettes, hence point of purchase merchandizing must be made effective if the soda fountain does the business expected of it.

Several examples are cited to show the effectiveness of "fruit window displays." Strawberries, cantaloupes and other fruits in season serve as effective display material, and it is claimed sales are materially increased accompanying their proper use.

Dramatization of fountain personnel, it is stated, is also an effective means of increasing sales. Life-sized pictures of fountain managers, or enlarged pictures of busy soda fountains can be used as centers of window displays to advantage according to the author.

Back bar displays of fruit in season serve as effective advertising it is stated. An outline is also given of the procedure used in planning and carrying through one of their "merchandizing drives."

W.C.C.

616. **Ice Cream is as Good as its Flavor.** E. G. WEED, Foote and Jenke, Inc. Ice Cream Field, 37, No. 5: 43. 1941.

The importance of properly selecting the mix ingredients is emphasized. No one flavor will blend with every type of mix according to the author and it is further stated that a mix with an "off" taste may need a small amount of "fortifier" in case of vanilla flavor, whereas a properly balanced mix with a good taste can be easily flavored with a minimum amount of pure, unfortified vanilla.

Since about 50 per cent of ice cream is flavored with vanilla, the selection of vanilla flavor is important. "Pure" vanilla can be obtained from many different sources and its quality also varies considerably. The increased use of vanilla concentrates is mentioned but it is stated that flavor losses vary from 10 to 30 per cent in the production of concentrated vanillas by heat processes. Mention is also made of vanilla powders prepared using (1)

finely ground beans mixed with sugar and (2) a mixture with or without beans but containing artificial vanillin.

New types of imitation vanilla flavors are being placed on the market, the author states, and he stresses the importance of buying from reputable manufacturers and having them use suitable specifications on labels.

Brief mention is made of fruit flavors including those from citrus products.

The proper care of flavors after purchase is essential if best results are to be expected, furthermore they should be used in the correct proportion it is pointed out.

W.C.C.

MILK

617. *Sur La Congélation du Lait. (On the Freezing of Milk.)* A. FOURNIER, the Sorbonne, Paris. *Le Lait*, 20: 390-402. 1940.

It is known that fresh meat withstands ordinary temperatures better than does meat which has been previously frozen. This difference is attributed to the fact that freezing destroys the cellular structure of the product.

Whether or not freezing alters the 'acidogenic' properties of milk and lactic ferments has been the subject of this study. Five ml. samples of varying proportions of milk and water, milk and lactic ferment, and lactic ferment and water have been prepared and the ability to produce acid after freezing, compared to unfrozen controls.

The ability to support acid production after freezing was not changed to what it was before being frozen. Dilution with water did not significantly affect the ability to support acid production. Although data are not given, it is stated that other properties such as flavor and reductase activity were not altered either. Apparently unsterile milk was used in this study since acidity developed spontaneously in all samples.

O.R.I.

618. *Conservation des Échantillons de Lait. (Preservation of Milk Samples.)* E. G. VOIRET AND BONAIME, Municipal Lam., Lyon. *Le Lait*, 20: 411-412. 1940.

The preservation of milk samples, especially in summer or in warm countries, presents many difficulties and many of the common preservatives are not entirely efficient. The use of amyl alcohol in conjunction with potassium dichromate appears to have great advantages as a preservative. The alcohol is a good antiseptic, and remains to a considerable extent in the cream layer. It is an emulsifying agent and aids in maintaining the original properties of the milk.

Its use is suggested at the rate of about one per cent alcohol to be added at the same time as the dichromate is added.

O.R.I.

619. *A New Method of Retarding Oxidized Flavor and Preserving Vitamin C—Deaeration.* PAUL F. SHARP, E. S. GUTHRIE, AND D.

B. HAND. Cornell University, Ithaca, N. Y. Internatl. Assoc. Milk Dealers, Assoc. Bul., 33rd yr., 20: 523-545. Apr. 1941.

Destruction of vitamin C and the development of oxidized flavor in pasteurized milk may be prevented by removal of the oxygen from the milk at the time of pasteurization. Milk at the time it is produced contains an average of 22.2 mgs. of vitamin C per liter but this has been reduced to 2.9 mgs. by the time the pasteurized milk is consumed. Mixed nights' and mornings' milk as delivered to the country milk plant was found to be 18.9 mg. per liter. Passage through the country milk plant did not decrease the vitamin C content much but increased the susceptibility to oxidation due to increases in copper and air content. Tank car transportation to New York City resulted in a further 3 to 5 mg. loss. At the city the cold milk was practically saturated with oxygen, the average being 10.3 mgs. per liter. During pasteurization by the holding method, 2 to 5 mgs. of vitamin C may disappear. When milk is heated over external surfaces, it loses some oxygen but takes more on when cooled over similar surfaces.

A commercial 3000 lb. per hour deaerator manufactured by the Thermal Engineering Corporation of Richmond, Virginia, is described. This is essentially a chamber in which the pressure is reduced to about .15 inches by means of 2 steam jets connected in series with an inner condenser. As the milk is introduced into the high vacuum chamber at 105° F. to 115° F. a drop in temperature of 7-15° F. occurs and the oxygen along with 0.5 per cent water vapor passes off as the milk boils. The total cost is 3 to 4 cents per 1000 lbs. of milk. True vacuum bottle fillers or bottom up type fillers are needed to prevent re-entry of oxygen. Milk with 19.4 mgs. of vitamin C per liter when aerated raw and pasteurized at 160° F. for 15 seconds showed 19.1 mgs. ascorbic acid after 3 days and no oxidized flavor. E.F.G.

620. **Remarks on Homogenized Milk.** IRVING J. WOLMAN, Children's Hospital of Philadelphia, Pa. Internatl. Assoc. Milk Dealers, Assoc. Bul., 33rd yr., 20: 546-550. Apr. 1941.

A group of 800 babies was divided into 4 groups of 200 fed on formulas made from: (1) Sonic vibration treated milk; (2) Milk homogenized at 2500 lbs.; (3) Milk homogenized at 750 lbs.; (4) Milk boiled for 5 minutes in the home.

All groups showed a similar growth curve and all milks seemed acceptable. Using homogenized milks simplifies the preparation of formulas but its selection over other available forms is a matter for the individual physician to decide. The dairy industry should establish standards for such milk which assure highest quality. E.F.G.

621. **The Value of Laboratory Control.** D. E. NOFSINGER, Richmond Dairy Co., Richmond, Va. *Milk Dealer*, 30, No. 7: 129-133. Apr. 1941.

A brief discussion of some of the laboratory tests used to control composition and quality is given. C.J.B.

622. **The Elwell Plan of Sliding Scale of Prices to Consumers.** EDWIN S. ELWELL, Northland Milk and Ice Cream Co., Minneapolis, Minn. *Internat. Assoc. Milk Dealers, Assoc. Bul.*, 33rd yr., 16: 411-417. March 1941.

The Elwell plan includes any systems in which additional quarts on the same delivery are sold for a lesser price than the first quart or the average price per quart of more than one quart is less than a single unit. Essentially the plan is a platform price to which is added a delivery charge, which is large on the first quart, but small or nothing on additional units. The purpose is to give the larger quantity buyer the benefit of savings in delivery. Since the retail price of milk is well below the delivered price of single units, the single quart customer can buy cheaper at the store but the multiple quart customer is more likely to give all business to the route man. Driver opposition to the plan has not been great because drivers realize that more drivers can be employed under this plan than if the customers are allowed to drift to milk depots and stores. Instead of presenting the plan to the customer as base price plus a delivery charge, it seems best to quote a price for the first unit, which includes delivery and additional units at a lower price. C.J.B.

623. **Chocolate Milks.** R. J. ALBERTS, Ohio State University. *Milk Dealer*, 30, No. 7: 126-127. Apr. 1941.

Following a brief discussion of the sales possibilities of chocolate milk, the author sets forth the following reasons for settling when non-settling chocolate is used:

1. Addition of the chocolate at too low or too high a temperature—manufacturers of chocolate specify a proper temperature and in the case of powders this is most generally 120-125° F. Some, however, prefer adding to the hot milk—150° F.—this is always so stated and must be followed for best results.
2. Old returns and high acid milk will cause settling besides giving a poor flavored chocolate milk—use only fresh dairy products.
3. Do not use cream and skim milk—use whole milk and standardize with fresh skim milk.
4. Too cold—less than 38° F. over surface cooler will cause too great a shock and will result in settling.

5. Too vigorous agitation—where high and low speed agitation is available on vat agitator, use only low speed.

6. Foaming—when using positive pump, always have line in back of pump full to prevent foaming.

7. Foaming—when using centrifugal pump, always keep flow to the pump full and regulate the speed of flow above pump.

8. Pre-cooling in vat is always dangerous although some chocolate manufacturers advise such to prevent too great a cooling shock at surface cooler. If pre-cooling takes too long (over five minutes) before chocolate milk is finally cooled, then it is too dangerous to attempt.

Other defects mentioned are: 1. Added viscosity because of too high a pasteurizing temperature. 2. Flocculating chocolate when chocolate milk is cooled too low—below 38° F. C.J.B.

624. Refrigeration Equipment for the Milk Dealer. I. A. MAHON, The Creamery Package Mfg. Co., Chicago. *Milk Dealer*, 30, No. 7: 41, 86-88. Apr. 1941.

The author points out that in dairy plants, volume is the measure of success and the bane of refrigeration designing. The very essence of volume makes for improbability, if not impossibility, of accurate refrigeration designing. Some suggestions are offered for installing refrigeration equipment which will more nearly meet the demand when the plant exceeds its planned capacity. C.J.B.

625. Refrigeration Equipment in Milk Production. H. O. ROBERTS, JR., Central Power and Light Co., Corpus Christi, Texas. *Milk Dealer*, 30, No. 7: 40, 83-86. Apr. 1941.

A brief review of the necessity for cooling milk followed by a discussion of how Texas dairymen are cooling their milk. It is pointed out that where electric service is available, most milk producers today are using some form of mechanical refrigeration. The rapid growth in the use of mechanical refrigeration by Texas dairymen is attributed largely to (1) capacity to cool milk rapidly, (2) saving in labor, (3) low cooling cost—lower electric rates, and (4) improved and lower-cost equipment. Current consumption for cooling milk on six representative farms in the Houston area, where milk was being cooled in tank type coolers and the evening milk was cooled and stored and the morning milk only cooled, showed that 0.98 kilowatt-hours was used per 100 pounds of milk cooled. C.J.B.

626. What Can Be Done to Improve the Quality of Milk. H. A. BENDIXEN, State College of Washington, Pullman, Wash. *Milk Dealer*, 30, No. 7: 38-39, 94-95. Apr. 1941.

The author states that in his mind the four cardinal requirements of a

high-quality market milk are that the milk be (1) nutritious, (2) palatable and of good keeping quality, (3) safe, and (4) clean and attractively packaged. The subject is then discussed under the following: 1. Can we improve the nutritive value of cow's milk? 2. Palatability and keeping quality. 3. Cleanliness and attractiveness in merchandising. C.J.B.

627. **That Cleaning Chore.** R. M. HOYT, Normal Sanitary Dairy, Normal, Ill. *Milk Dealer*, 30, No. 7: 30-31, 75-78. Apr. 1941.

The necessity of using the proper washing powders, temperatures, etc., in the cleaning operations of a milk plant are discussed. Special emphasis is placed on the necessity of properly training new employees. C.J.B.

628. **Engineering Features of Pasteurizing Plants and Equipment.** G. A. A. BURN, Ontario Dept. of Health, Toronto. *Canad. Pub. Health Jour.*, 32: 199-207. 1941.

Ontario's regulations for milk pasteurization plants are interpreted by a sanitary engineer, special attention being given to plant construction, milk processing equipment and the correction of defects in pasteurization systems. The use of high-short pasteurization is not, as yet, a legal method for fluid milk in Ontario. O.R.I.

629. **A Laboratory Procedure for Detecting and Eliminating Thermophilic Bacteria from Pasteurized Milk.** V. E. GRAHAM AND W. H. ORME, Univ. of Saskatchewan. *Canad. Pub. Health Jour.*, 32: 70-71. 1941.

This procedure consists of two steps, the first to check the efficiency of pasteurization in the plant being inspected and the second to detect the offending shipper.

In the investigation of an outbreak, samples should be taken from all vats in the plant at the conclusion of the holding process and before cooling. This sample is cooled in the sample bottle and taken to the laboratory. Here, the sample is divided and a portion repasteurized. Both the repasteurized and original samples are then replated. Several dilutions should be used and incubation should be at 37° C.

If thermophilic organisms are present there will be little or no reduction in the count on the plates from the repasteurized sample. In such cases individual samples are taken from each producer's milk and part of each of these subjected to laboratory pasteurization. Plates are then prepared using a dilution of 1:1000 for the raw milk and 1:100 for the pasteurized. The presence of thermophilic organisms will be indicated by a high count on the plates from the pasteurized samples. In such cases, further work will have to be done on the farm and improperly cared for milking machines are often the cause of the trouble. O.R.I.

630. **Milk Consumption in the Vancouver Metropolitan Area.** J. S. KITCHING, Vancouver, B. C. *Canad. Pub. Health Jour.*, 32: 154-157. 1941.

By means of a questionnaire circulated to 16,000 homes in the Vancouver district, a picture was obtained of the milk consuming habits of a large section of the community. The average per capita consumption was calculated to be 0.83 pints per day. Many of the factors affecting consumption are similar to those reported in other surveys of this kind. O.R.I.

631. **Consumption of Milk in Canada.** W. C. HOPPER, Dominion Dept. Agr., Ottawa. *Canad. Pub. Health Jour.*, 32: 147-153. 1941.

Statistics are given for per capita consumption of fluid milk in different sections of Canada for different racial, income, occupational and age groups. The figures are for fresh fluid milk and do not include that purchased in other products. For most cities the average was approximately 0.70 Imperial pints per day. French-Canadians had a lower per capita consumption than those of other groups as did Jews, Italians and Orientals. Consumption by farm families was over one pint while in all groups consumption increased as did family income. Only 23 per cent of the adults (over 16 years) drink any milk. The proportion of adults drinking milk increased as family income increased.

Children of relief and low income families in cities more frequently were milk drinkers than were children in families of higher income groups. O.R.I.

632. **The Application of the Evelyn Photo-Electric Colorimeter to a Modification of Kay and Graham's Phosphatase Test.** J. WYLLIE, Queen's Univ., Kingston, Ontario. *Canad. Pub. Health Jour.*, 32: 122-128. 1941.

A modification of the original Kay and Graham test is proposed in which the color produced is more accurately measured in a colorimeter. Standard colorimeter values for this instrument are suggested for both A and B tests. It would seem, however, that the standards adopted are such as to allow under-pasteurized milk to be passed. The method affects a saving through the use of smaller amounts of reagents. O.R.I.

633. **Practical Experience with the New Medium in Quality Control.** A. J. POWERS, Borden's Farm Products, Brooklyn, N. Y. *Internatl. Assoc. Milk Dealers, Assoc. Bul.*, 33rd yr., 17: 443-459. March 1941.

Results with the new tryptone-glucose-extract-milk agar over a period of eighteen months have revealed the following. Comparing the counts obtained with the new medium in the first year of its use with the counts

obtained on the old nutrient agar in the preceding year, Grade A milk showed in monthly average counts 1.03 to 4.26 times higher with the new medium, grade B milk 2.00 to 5.13 times higher and pasteurized cream 0.76 to 7.80 times higher. Weekly averages of parallel counts on grade A pasteurized milk were 1.08 to 8.0 times higher for the new medium and grade B pasteurized 1.08 to 5.24 times higher. Interest in "pasteurizability" and study of the problem of individual farm samples have indicated clearly that proper sanitary practice at the individual dairies or the receiving plants generally removes the tendency toward high counts after pasteurization.

Replies to a questionnaire to 34 industry laboratories with regard to their experience with the new medium are summarized. The use of the new medium has encouraged the pasteurization of individual dairy samples to locate the source of milk difficult to pasteurize satisfactorily. It is thought that the new medium has made the plate count a more useful tool in milk control work.

E.F.G.

634. **Introducing a Selection System for Milk Route Salesmen, Clerks, and Other Personnel.** VERNE STEWARD, Los Angeles, Calif. Internatl. Assoc. Milk Dealers, Assoc. Bul., 33rd yr., 18: 467-471. Apr. 1941.

A description and explanation are given of the I.A.M.D. manual entitled "How to Appraise Prospective Milk Route Salesmen" also the "Composite Inventory and Examination for Milk Industry Employees" and the "Medical Examination Form."

Careful use of the above helps is recommended in order that the waste from employing men not suited to this sort of work may be avoided. Attention is called to the fact that persons ill-suited to the work of route salesmen are eliminated with greater difficulty and expense than formerly.

E.F.G.

635. **Cost Reports for the Plant Manager.** BRUCE BALDWIN, Baldwin Dairies, Inc., Philadelphia, Pa. Internatl. Assoc. Milk Dealers, Assoc. Bul., 33rd yr., 16: 435-439. March 1941.

Some of the more valuable reports are: product cost reports, milk fat losses, fluid milk losses, fluid cream losses, manufacturing losses, bottle losses, power fuel and light costs, washing powder cost and labor costs. The point is made that the selection of the basis and the form of these reports should tend toward simplicity and ease of understanding if the hoped for benefits are to be realized.

E.F.G.

636. **Cost Reports for Sales Managers.** F. W. ROOT, Glendale Farms, Inc., Wilkes Barre, Pa. Internatl. Assoc. Milk Dealers, Assoc. Bul., 33rd yr., 16: 431-434. March 1941.

The uses to which the sales manager can put certain reports as overhead statements on selling and delivery costs, individual route costs, and individual truck costs are described. Individual route cost reports are considered to be the most valuable.

E.F.G.

637. **The Single Service Container and Its Effect upon Milk Distribution Costs.** E. L. VEHLLOW, Calif. State Dept. Agr., Sacramento, Calif. Internatl. Assoc. Milk Dealers, Assoc. Bul., 33rd yr., 16: 418-430. March 1941.

Prior to 1939 only fibre containers sterilized on the premises were permitted. Since that time factory formed containers have been permitted so that by August 1940 nearly 50 per cent of all milk sold to consumers through retail stores in the major California milk markets where fibre has been introduced is in fibre containers.

Added processing cost over glass ranged from .86 cent to 1.15 cent for various types of fibre containers. If the cost of the fibre container is to approach the cost of glass operation it must come from greater efficiency in delivery. The most efficient fibre operator delivered milk for .5042 of a cent less than the most efficient exclusive glass container operator. The net added cost of fibre over glass was .3544 of a cent per quart. The elimination of the bottle deposit results in glass container costs comparable with fibre. Grocer preference has boosted the sale of milk in fibre containers. Many distributors have reduced the fat content of milk in fibre containers close to the legal limit to compensate for the added cost of the fibre container. An added one-half cent wholesale for milk in fiber containers in Los Angeles has not been passed on to the consumer but absorbed by the retailer.

E.F.G.

638. **Fluid Milk Production Costs.** PAUL YOUNG, Telling Belle Vernon Co., Cleveland, Ohio. Milk Dealer, 30, No. 6: 116-120. March 1941.

A discussion is presented of the extra costs of requirements for the production of fluid market milk over milk for manufacturing purposes. The author suggests that the problem of increasing consumption and of determining a fair remuneration to producers for the extra costs of supplying milk for fluid markets, could be solved most satisfactorily by giving careful study to the individual problems peculiar to every market and then basing fluid milk producer prices on the evaporated code price plus the extra costs of meeting fluid milk quality, quantity, and transportation requirements above evaporated requirements.

C.J.B.

639. **Comparison of Chemical, Steam and Hot Air Sterilization of Dairy Equipment.** HARRY H. WEISER, Ohio State University. Milk Dealer, 30, No. 6: 112-114. March 1941.

A discussion of the sterilization of dairy equipment, in which data are presented to show the effect of varying the pH, temperature, and time on the germicidal effect of a chlorine solution. Data are also presented to show the germicidal effect of alkyl-aryl-sulfonate when added to the chlorine solution. The following general observations are made by the author :

1. Various types of washing solutions are effective detergents in cleaning milk utensils and dairy equipment but are not efficient sterilizing agents.

2. Chlorine compounds are efficient and practical sterilizing agents when properly handled.

3. A chlorine solution adjusted to pH 6.0 increases the germicidal power of the compound. However, an acid solution of the compound is less stable and has a greater corrosive action on the equipment than an alkaline chlorine solution.

4. Chlorine compounds are less effective as a sterilizing agent in the presence of organic matter.

5. Relatively low concentrations of chlorine are effective in destroying bacteria in the absence of organic matter.

6. A temperature range from 50° F. to 90° F. showed very little difference in the germicidal efficiency in the pH value of the chlorine solutions.

7. The addition of alkyl-aryl-sulfonate to the chlorine solution enhanced the germicidal efficiency of the chlorine compounds.

8. The use of washing solutions combined with the use of steam as a sterilizing agent is effective in sterilizing milk cans, etc., followed by currents of hot air to dry the can.

9. The use of dry heat for sterilizing laboratory glassware is satisfactory.
C.J.B.

640. **Suggestions on How to Make Good Buttermilk.** A. D. BURKE, Alabama Polytechnic Institute. *Milk Dealer*, 30, No. 6: 41, 82-83. March 1941.

The essentials of making good buttermilk are discussed from the following standpoints: 1. Quality of milk used. 2. Selection of cultures. 3. Preparation of mother culture milk. 4. Sterilization. 5. Temperature control. 6. The bulk product. 7. Culturing the bulk product. 8. Breaking the curd.
C.J.B.

641. **Tips on Cleaning Test Bottles.** LEONARD BEACH, Beach Milk Co., Denver, Col. *Milk Dealer*, 30, No. 6: 35, 46. March 1941.

The method of washing test bottles used at the Beach Milk Co. is as follows: 1. Select a good washing powder. This plant uses tri-sodium phosphate, because of its rinsing properties. 2. Place just enough dry powder in each test bottle to cover the bottom. 3. Add enough cold water to form a thick paste. 4. Shake bottles; a few quick shakes should be enough. 5.

Rinse with cold water. The cold water is important if tri-sodium phosphate is used for a washing powder. A diagram of a special apparatus for rinsing the bottles is given. It consists of a small copper tube connected to the cold water supply, sealed at the end and with holes in the side.

C.J.B.

642. **Milkstone Formation.** LEWIS SHERE, Diversey Corp., Chicago. Milk Dealer, 30, No. 6: 33, 56-64. March 1941.

A discussion is given of the factors causing and methods of preventing milkstone formation. Methods of removing milkstone which has formed on equipment are also discussed.

C.J.B.

643. **Six Day Delivery in Canton and Akron, Ohio.** ANONYMOUS. Milk Dealer, 30, No. 6: 31, 77-78. March 1941.

A general discussion is given of dealer, employee, and public reaction to six-day delivery of milk in Canton and Akron, Ohio.

C.J.B.

644. **Six Day Milk Delivery—Six Day Delivery from the Plant Angle.** H. D. DRAIN, Peoples Dairy Co., Akron, Ohio. Milk Dealer, 30, No. 6: 31, 78-79. March 1941.

The author discusses the following problems which confronted the plant in the change to a six day delivery: 1. Provision for a supply of fluid milk available for needs of the sales department. 2. Selection of a day the plant should operate on a reduced schedule. 3. Supply and storage for cases and containers. 4. Storage room for finished products. 5. Ways of handling returns. 6. Product difficulties.

It is concluded that all of the problems involving the plant can be handled in a reasonably satisfactory manner. Some savings in labor, power, light, and water are possible.

C.J.B.

645. **Six Day Milk Delivery—Columbus Humanizes the Marketing of Milk.** J. C. NISBET, Ohio Dairy Products Assoc. Milk Dealer, 30, No. 6: 30, 75-76. March 1941.

The reaction of the public to daylight, no-Sunday delivery of milk and its effect on sales and labor are discussed. Summarizing the proposition of how to sell no-Sunday, daylight delivery, the author states the Columbus experiment would emphasize the following:

"First, to point out that the great improvements made in the production, processing and distribution of milk have eliminated the necessity for daily delivery by eliminating the problem of keeping milk an extra day—or several days for that matter.

"Secondly, eliminate entire 'time of delivery' as a competitive argument. Convince your customer that whenever you deliver the milk, whether

it be 8 A.M. or 12 noon, that she will receive a 24-hour supply. All she need do is purchase the extra amount needed for the first day and an extra day's supply each Saturday.

"Remember, whenever anything new is introduced that changes habits of long standing, there may be some confusion during the period of adjustment. Expect this. Anticipate it and be prepared, but recognize that such a period will soon be over and the benefits will start to accrue."

C.J.B.

646. **For Precise Reading of Babcock Test.** E. O. HERREID, Vermont Agr. Expt. Sta., Burlington. Natl. Butter and Cheese Jour., 32, No. 6: 30. 1941.

A piece of equipment invented by Julius Hortvet for measuring the length of the fat column has been improved. Estimations can easily be made to 0.025 per cent. This equipment is not being manufactured at the present time. W.V.P.

647. **Practical Pointers on Quality Production.** DAVE NUSBAUM, Univ. of Wisconsin, Madison. Natl. Butter and Cheese Jour., 32, No. 6: 18. 1941.

Quality of dairy products is lowered by off-flavors and odors caused by bacterial growth. Such growth in milk depends on contamination, food, water, time, temperature and other essential factors such as oxygen tension and pH which cannot be readily discussed with milk producers. Control of contamination can be accomplished by careful farm practices such as clean barns, plenty of bedding, clipping of udders, cleaning of cows before milking, proper maintenance, cleaning and sterilizing of utensils. Prompt cooling of milk is essential. Keep milk Clean, Cool and Covered. W.V.P.

648. **Survey of Milk Control, Including the Extent of Pasteurization in Municipalities of Two Thousand Population in Canada. Survey of Milk-Borne Diseases in Canada.** Milk Committee of the Canad. Pub. Health Assoc. Canad. Pub. Health Jour., 32, 216-226. 1941.

Statistics are presented relating to licensing, inspection, tuberculosis and contagious abortion testing and extent of pasteurization for most towns and cities in Canada.

A survey of all the milk-borne types of diseases recorded in Canadian municipalities between 1912-1940 is also presented. O.R.I.

649. **Progress in Pasteurization in Ontario.** A. E. BERRY, Ontario Dept. of Health, Toronto. Canad. Pub. Health Jour., 32: 208-212. 1941.

Since 1938 Ontario has made compulsory the pasteurization of milk in all cities and towns and has gradually extended these regulations to cover villages and some unincorporated areas and summer resorts. It is now estimated that 98 per cent of the fluid milk sold is pasteurized.

Public health statistics for 1939 show a 45 per cent reduction in undulant fever cases and the typhoid fever death rate was lowered about 50 per cent. Paratyphoid and infant mortality were substantially reduced. It is reasonable to contend that pasteurization was partly responsible. O.R.I.

PHYSIOLOGY

650. **Growth of the Mammary Gland Following Local Application of Estrogenic Hormone.** WARREN O. NELSON, Dept. of Anatomy, Wayne Univ., Detroit, Mich. Amer. Physiol. Soc. Proc., p. 209. Apr. 1941.

The theory that the ovarian hormones stimulate growth of the mammary glands only through their action on the anterior hypophysis and the production therein of one or more mammogenic hormones is controverted by experiments in which application of estrin to the area of one gland has induced growth of that gland only (women, monkeys, rabbits). The present work deals with similar studies in the guinea pig.

Seven gonadectomized male and female guinea pigs received applications of estrin to one mammary gland area. In three animals the second nipple was massaged daily with sesame oil alone.

Growth of the nipple on the side receiving applications of estrin was evident by the 8th day and continued throughout the period of treatment in each animal.

In only one instance was any growth observed in the nipple on the side receiving sesame oil only. The mammary glands recovered from the side treated with estrone showed development of ducts and buds at 15 days, and progressive growth of both ducts and alveoli at 25 and 35 days. A slight growth of ducts was produced in one gland which received applications of oil only.

These results present further evidence that local application of small amounts of estrin to the area of a mammary gland will stimulate growth of the gland only. D.L.E.

651. **The Lack of Inactivation of Stilbestrol by the Liver.** M. J. ALLEN, Northwestern Univ. Med. School, Chicago. Am. Physiol. Soc. Proc., p. 6. April 1941.

Stilbestrol differs from the natural estrogens in that it is very potent by mouth. There is good evidence that natural estrogens are inactivated by

the liver. Other workers have reported that when ovaries are transplanted intrasenterically in such a position that their venous drainage is into the portal system, they have no estrogenic effect. This liver inactivation is considered to be the reason why natural estrogens are relatively ineffective by mouth. It was considered possible that the high oral potency of stilbestrol might be due to the inability of the liver to inactivate it.

This hypothesis was tested. Stilbestrol pellets (approximately 3.0 mgm. each) were implanted intrasenterically into 10 castrate female rats and subcutaneously into 10 control castrate rats. After 48 hours the animals of both groups went into prolonged estrus. It is concluded that stilbestrol differs from natural estrogens in that stilbestrol is not inactivated by the liver. It seems probable that this fact explains the oral potency of stilbestrol.

D.L.E.

652. The Blood Precursors of the Short Chain Fatty Acids of Milk. J. C.

SHAW AND C. B. KNOTT, Dept. Dairy Industry, Storrs Agr. Exp. Sta., Storrs, Conn. Am. Physiol. Soc. Proc., p. 255. Apr. 1941.

Arteriovenous differences demonstrated that acetone bodies were used by the lactating gland of the cow. Fractionations disclosed that the utilization of acetone bodies was limited to β -hydroxybutyric acid. According to arteriovenous differences the quantity of β -hydroxybutyric acid utilized is just sufficient to provide for the fatty acids C_{14} and lower. Such synthesis would explain the high R.Q. of the lactating gland. Approximately 40 per cent of the total oxygen consumption of the gland would be required for the complete oxidation of β -hydroxybutyric acid for energy purposes. Considering the high R.Q. of the normal lactating gland such a postulation does not appear to be warranted.

The R.Q. of the lactating gland in periods of inanition and cod liver oil feeding was less than unity. Likewise marked decreases in Reichert-Meissl values of the milk fat occurred in both cases. Simultaneously the β -hydroxybutyric acid arteriovenous differences decreased significantly. When dextrose was fed in large quantities or pumped into the rumen there was a fall in blood acetone bodies of over 50 per cent which resulted in a decrease in the arteriovenous difference of β -hydroxybutyric acid. Coincident with the decline in blood β -hydroxybutyric acid there was a marked decrease in the saponification number, Reichert-Meissl value and Polenske value of the milk fat.

In severe ketosis in dairy cows in which both blood glucose and blood lactic acid were less than 50 per cent of normal, the R.Q. of the active gland was in excess of unity and indicated that carbohydrate material is probably not used for fat synthesis in the gland.

It is concluded that β -hydroxybutyric acid is probably used in the synthesis of the short chain fatty acids of milk.

D.L.E.

653. **Desoxycorticosterone and Lactation.** ROBERT GAUNT. Dept. of Biology, New York University. Am. Physiol. Soc. Proc., p. 101. Apr. 1941.

This report concerns the effect of desoxycorticosterone acetate (DCA) on the lactation of rats adrenalectomized within 24 hours after delivery.

When the mothers received 0.3–0.5 mgm. DCA per day all the young died between the 11th and 19th days, despite large weight gains and excellent health of the mothers (8 litters). One-tenth milligram DCA gave similar results in 3 cases, but one anomalous animal lactated normally.

This would seem to indicate that DCA not only failed to support lactation but probably actually inhibited it, perhaps because it is a mammary-growth stimulating substance.

DCA did not, however, inhibit lactation of intact rats in doses of 0.5 mgm. per day (3 litters), showing that if there is an inhibitory effect it can be prevented (at this dose level) by the normal cortical secretions.

This was further illustrated by giving 0.3 mgm. DCA per day plus 2 cc. Eschatin to the adrenalectomized mothers of 4 litters. All the young were raised to weaning although growth was not normal.

Although these results might be interpreted as indicating that DCA, due to its lack of ability to maintain a normal carbohydrate metabolism or for some analogous reason, is qualitatively incapable of sustaining lactation, such an interpretation is not necessary until the possibility that it acts as a direct lactation inhibitor is more completely ruled out. D.L.E.

MISCELLANEOUS

654. **Water Supply and Sewage Disposal for Dairies.** J. R. FLEMING, Univ. of Tennessee. Milk Dealer, 30, No. 7: 52–54. Apr. 1941.

Water supplies are discussed as to (1) availability, (2) adequacy, and (3) quality. Sewage disposal is discussed mainly from the angle that it shall not contaminate the water supply. C.J.B.

655. **Recommended Methods for Cleaning and Sterilizing Stainless Steel Equipment.** ANONYMOUS. Milk Dealer, 30, No. 7: 72. Apr. 1941.

The methods for cleaning and sterilizing stainless steel equipment, issued by the Alloy Tank Manufacturers Council and the Batch Pasteurizer Manufacturers Council, and approved by the Technical Committee of the Dairy Industries Supply Association, Incorporated, are given. C.J.B.

656. **What Should Go in the Advertising Appropriation.** P. H. KEMPER, Bowman Dairy Co., Chicago, Ill. Internat'l. Assoc. Milk Dealers, Assoc. Bul., 33rd yr., 18: 472–478. Apr. 1941.

The method recommended is not based on a percentage of sales, a unit of sale method or proportion of profits but is based upon an elastic budget, used where it will do the most good and from which unwarranted charges are excluded. There is much disagreement among companies as to which items are properly charged to advertising and which should be charged to some other account. Reports from 62 companies showed 1.29 per cent of the sales' dollar going into advertising. E.F.G.

657. The Frozen Food Industry. HARRY CARLTON, Univ. Tenn. Knoxville, Tenn. Agr. Expt. Sta. Bul. 173.

This is a very comprehensive bulletin of 175 pages dealing with the fast growing frozen food industry. The bulletin, practically a text book on the subject, is divided into four parts as follows: Part I. Distribution; Part II. Production for freezing; Part III. Processing operations; Part IV. Miscellaneous.

Part I covers early history, early distribution difficulties, growth of quick-frozen food distributions, channels of distribution, increased acceptance, frozen-food production, markets, sales policies, retail cabinets, selling prices and losses.

Part II discusses freezing operations, variety of fruits and vegetables, packing areas, seasons for packing, leading fruits used, yields, prices paid grower and harvesting costs.

Part III deals with quality control, vitamin content of quick-frozen food, blanching for quick frozen foods, fine modern methods used in freezing, packaging frozen foods, cost of packaging, costs comparison between quick-freezing and canning costs.

Part IV covers such subjects as cold storage rates in leading fruit and vegetable packing areas, transportation rates for frozen fruits and vegetables, quick-frozen poultry, freezer locker plants and their operation.

The bulletin treats the subject very completely and is a decided contribution to the frozen food industry and in addition to the above subjects contains many statistics of this important industry. C.D.D.

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ABSTRACTS OF LITERATURE

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New York Association of Dairy and Milk Inspectors	United States Department of Agriculture

ABSTRACTS OF LITERATURE

ADVANCE ABSTRACTS OF REPORTS ACCEPTED FOR PUBLICATION

658. **The Bacteriology of Brick Cheese. I. Growth and Activity of Starter Bacteria.** JOHN C. GAREY, EDWIN M. FOSTER AND WILLIAM C. FRAZER, Department of Agricultural Bacteriology, University of Wisconsin.

Brick cheese was manufactured by the conventional method with *Streptococcus lactis* and *Streptococcus thermophilus* starters used singly and in different combinations. The growth and activity of the starters were followed by bacteriological, chemical and physical methods.

When 0.6 per cent of *Str. lactis* starter was used alone and the curd cooked to 106° F., the development of the lactic streptococcus was very slow until the third or fourth hour after dipping; thereafter the numbers increased rapidly and reached their maximum at one to two days. If the curd was cooked at 112° F., the growth of the *Str. lactis* bacteria was decreased as evidenced by a slower rate of multiplication and a lower maximum number. Because of the lack of activity of the lactic streptococcus until the latter part of the draining, it was necessary to dip the curd in a relatively dry condition otherwise the cheese would retain too much moisture and those defects characteristic of an acid cheese would develop.

When 0.6 per cent *Str. thermophilus* starter was used alone and the curd cooked to 106° F., growth of the starter bacteria was most rapid during the cooking and the first three hours after dipping; thereafter the rate of multiplication decreased sharply because of the unfavorably low temperature in the cheese. A cooking temperature of 112° F., in comparison with 106° F., increased the growth and activity of the thermophilic streptococcus. When *Str. thermophilus* starter was used alone, all the lactose was not fermented in the cheese. This was evident from the high pH at one day, the later development of *Str. lactis* in large numbers and the development of undesirable bacteria which produced gassiness and fermented flavors in the cheese.

Alone, neither of the starters produced a Brick cheese of satisfactory quality. The cheese manufactured with *Str. lactis* starter developed a sour flavor and a short and crumbly body, that with *Str. thermophilus*, a fermented flavor and a very open texture. Of the different combinations of starters tried (cooking temperature—106° F.), a mixture of 0.3 per cent each of *Str. lactis* and *Str. thermophilus* produced a Brick cheese of more desirable quality than that with the other combinations.

If the moisture was higher than 42 per cent after salting, it was a practical guarantee of an acid or sour cheese with characteristic defect. This

meant that the moisture of the ripened cheese had to be 2 to 4 per cent lower than the legal limit in order to produce a desirable Brick cheese.

659. Lipolytic and Proteolytic Activities of Various Penicillia. C. JENSEN, North Dakota Agricultural Experiment Station, Fargo, North Dakota.

Studies were made of the lipolytic and proteolytic activities of the penicillia employed in the ripening of blue veined cheeses. The report deals with a study of 23 strains of penicillia including one *P. chrysogenum*, 3 *P. gorgonzola*, 15 *P. roqueforti*, one *P. stilton* and 3 unidentified strains.

There was considerable variation in the lipolytic activities of various penicillia on butterfat and cottonseed oil, as determined by the Nile blue sulfate technique; the intensity and uniformity of lipolysis of the cultures ranged from nonlipolytic to very pronounced lipolytic.

There was considerable variation in the lipolytic activities of various penicillia on different triglycerides according to the Nile blue sulfate technique. Only a few cultures hydrolyzed tripropionin, while all readily hydrolyzed tributyrin and trivalerin. As the molecular weights of the triglycerides increased, variation in lipolytic activities became more conspicuous. Some cultures showed gradual declines in their lipolytic activities, whereas others declined sharply on the triglycerides beginning with tricaproin.

There was considerable variation in the toxic effect of different triglycerides on various penicillia. In general, the triglycerides that exhibited the most pronounced toxicity, in declining order of their effect, were tripropionin, tributyrin, trivalerin, tricaproin, trilaurin, trimyristin and tripalmitin. The least toxic were triheptylin, tricaproin and triolein.

There was considerable variation in the proteolytic activities of various penicillia as determined by the acidified milk agar and the carbon dioxide techniques. There was a general agreement between the results obtained with the two techniques.

The rates of growth and of proteolysis of certain penicillia were affected by different growth conditions. The cultures grew more slowly but showed greater proteolytic activities in air at 28° C. than at 19° or 12° C.; the cultures were somewhat retarded in growth but proteolysis was unaffected when grown at 28° C. in an atmosphere in which 10 per cent of the air had been replaced by carbon dioxide; culture growth and proteolysis at 28° C. were almost stopped in an atmosphere saturated with carbon dioxide; growth usually was unaffected but proteolysis was accelerated at 28° C. in an atmosphere consisting for the most part of nitrogen.

660. A Test for the Protein Stability of Milk. ARNOLD B. STORRS, American Seal-Kap Corporation, Long Island City, N. Y.

A test for protein stability which requires a minimum of equipment, reagents and technical skill is described. Increasing amounts of N/10 HCl

are added to 10-ml. portions of the milk to be tested. The mixtures are placed in a boiling water bath for 10 minutes and then examined for coagulation. The stability number is equivalent to $100 \times$ the ml. of HCl required to produce coagulation under the conditions of the test.

The average stability number of untreated fresh milk has been found to be about 60 to 70 as indicated by the test. Pasteurization tends to increase the protein stability of milk while copper contamination tends to lower the stability.

661. **A Method for the Estimation of Nicotinic Acid in Milk.** E. A. BAILEY, JR., W. J. DANN, G. HOWARD SATTERFIELD, AND C. D. GRINNELLS, Duke University and North Carolina State College of Agriculture.

A chemical method for the estimation of nicotinic acid, the pellagra-preventive factor, in milk is reported. The essentials of the method are: Acid hydrolysis, removal of interfering salts and colored material by treatment with Lloyd's reagent and lead hydroxide, and development of a colored complex by treatment with cyanogen bromide and metol (p-methylamino-phenol sulfate). The reproducibility of results and the recovery of added nicotinic acid by this method are found to be satisfactory.

The analysis of milk from Ayrshire cows during the month of January 1941, shows the normal nicotinic acid content of the milk from these cows during this period to have been 1.46 micrograms per ml.

The low value obtained for the nicotinic acid content of milk is of interest in view of the fact that milk has long been considered of great importance in pellagra-preventive diets and has been shown to have pellagra-preventive value. The possibility is suggested that when considerable quantities of milk are included in the diet, the intestinal flora are so altered that a significant amount of nicotinic acid is synthesized by the intestinal microorganisms.

662. **The Effect of the Administration of Shark Liver Oil on the Butter Fat and Milk Production of Cows.** H. J. DEUEL, JR., Department of Biochemistry, University of Southern California Medical School, Los Angeles.

Shark liver oil was administered to six Guernsey cows in amounts of 30 cc. daily (700,000 I.U. of vitamin A) and a comparison in milk production was made with that of six other cows receiving the same basal diet without the supplement. All cows received the same basal diet which included large amounts of fresh-cut alfalfa. In a five-week preliminary period, the average milk production was practically identical in the two groups. With the feeding of the shark liver oil, an immediate rise in milk production of approximately 10 per cent over the control level was noted which continued

for 11 weeks; this gradually increased to a value of over 20 per cent by the 16th week of the test. An increase in butter fat given by the cows receiving the oil supplement over that of the control animals varied during this period between 507 and 794 grams per week per cow. These alterations are not ascribable to season, lactation cycle, or to food and are believed to be caused by the administration of the shark liver oil.

663. **Age, Live Weight and Milk-Energy Yield—A Correction.** W. L. GAINES, C. S. RHODE AND J. G. CASH, University of Illinois.

This correction removes certain systematic errors in the live weight estimates of a previous paper (this journal Oct., 1940). The most important change thereby effected is that the new (more correct) live weight data show that within herd milk-energy yield is proportional to the 1.02 power of live weight in the Holstein records; and proportional to the 0.98 power of live weight in the Jersey records. Live weight is measured within the first 31 days after calving and yield is for the first 8 months of the lactation.

BACTERIOLOGY

664. **The Bacteriological Analysis of Creamery Waters.** H. WOLOCHOW, Science Service, Dept. of Agr., Ottawa, Ont.; H. R. THORNTON, Univ. Alberta, Edmonton, Alberta, AND E. G. WOOD, Science Service, Dept. of Agr., Ottawa, Ont. *Canad. Dairy and Ice Cream Jour.*, 20, No. 2: 23. 1941.

Creameries in many parts of Canada have experienced very serious trouble in the past few years from butter deteriorations caused by the use of contaminated water supplies, although in many cases these waters were of potable standard. Eighty-five samples of water from 37 Alberta creameries were examined for specific types of organisms. A surprising number of the waters contained large numbers of bacteria capable of growth at 10° to 15° C. Many of the bacteria were proteolytic according to the criteria applied. Further investigation may indicate the necessity of stricter bactericidal treatment of all creamery waters. O.F.G.

665. **Burri Technique Discussed.** H. H. WEISER, Ohio State Univ., Columbus, Ohio. *Amer. Milk Rev.*, 2, No. 7: 157-158. July, 1940.

The Burri agar slant method offers an inexpensive, rapid, simple procedure for determining relative numbers of bacteria in milk, and may be modified for use with butter, cheese, and ice cream. The original method has been considerably modified. Directions for making the test are given. The method gives better differentiation of colonies than the ordinary plate method because all colonies are on the surface; the count is lower because

clumps are not completely broken up; composition of the medium may be varied to suit the growth of particular types of bacteria; and it offers a good means for cultural examination of mastitis milk. P.S.L.

666. Heat Resistant Bacteria. A. C. MAACK. Amer. Milk Rev., 3, No. 1: 1-2. Jan., 1941.

Survival of heat resistant bacteria after pasteurization presents a serious problem in attempts to regulate plate counts of pasteurized milk. Both thermophilic and thermoduring bacteria compose the groups surviving, and both rod and spherical forms are found. Feed, bedding, and soil harbor thermophilic organisms and utensils and milking machines often are a source of thermoduric types. Milk from suspected farms may be plated to locate sources. Exposure to chlorine of 100 p.p.m. for 2 or 3 minutes is necessary to kill these organisms. Repasteurization is often a cause of trouble as is incomplete pasteurization of foam, and milk stone in vats is strongly suspected as acting to harbor bacteria of these strains. In controlling infection all utensils must be sterilized. Infections are more common during cold weather when carelessness in cooling is more apt to occur. P.S.L.

667. Is Zero To Be the Limit. M. E. PARKER. Amer. Milk Rev., 3, No. 6: 128-130. June, 1941.

In this article the author has reviewed action of the Coordinating Committee on Standard Methods of the American Public Health Association to better the method of making plate counts of milk. The author suggests that emphasis be placed on qualitative rather than quantitative methods for evaluating quality of milk. He believes that such quantitative emphasis will result in lowered palatability, that it will gradually reach a point where cost would not be justified by the safety attained, and that the plate count for evaluating quality is futile and confusing. Adoption of cultural media designed to develop the maximum number of bacteria does not give a true picture of the numbers of bacteria in milk causing spoilage. It will cause reduction of counts by increasing the care given to milk production but will not necessarily increase quality. Lowering bacterial counts has increased susceptibility to oxidized flavor in milk and surface taint in butter, due to the upsetting of the balance in the normal bacterial flora. If the goal is zero it means reduction in quality with consequent reduction in consumption. P.S.L.

BREEDING

668. Eine volle Laktationsperiode umfassende Amidfütterungsversuche mit eineiigen Rinderzwillingen. J. SCHMIDT AND J. KLEISCH, Univ. Berlin. Züchtungskunde, 15, No. 16: 169-174.

A pair of identical twin heifers were fed through a whole lactation period

with one of them receiving in the form of amide nitrogen almost half of the protein judged necessary by present feeding standards. The fat percentage was identical and the production for the heifer receiving the amide nitrogen was 3755 kg. of milk and 126.5 kg. of fat, as compared with 3936 kg. of milk and 132.6 kg. of fat for the other heifer. No differences in the general health of the animals were observed. J.L.L.

669. Vergleichende Untersuchungen über den Zusammenhang zwischen Alter und Leistung bei verschiedenen Rinderrassen, durchgeführt an Kühen des Deutschen Rinderleistungsbuches (RL). J. LANGE, Univ. Berlin. Züchtungskunde, 16, No. 4: 123-126. 1941.

Whether dairy breeds differ in the regression of milk and fat production on age was studied on data from the German "Rinderleistungsbuch." These data correspond somewhat to the data from the Herd Improvement Registry testing in the United States, except that the German data are reported by association testing year instead of lactations, and the cows are selected cows. The lifetime records of 2,113 cows of the lowland races and 452 cows of the highland breeds of Germany were studied. Each of these cows had at least 7 years of records. By this restriction it was thought that the effects of selection on the age curve would be avoided, but the possibility of the opposite error arising because of the imperfect repeatability of the records on which past selection may have been based is not considered.

In general the breed-to-breed differences were small and it is doubtful whether they were statistically significant. The middle German red breed did not decline as rapidly after maturity as the others and this is attributed to innate resistance and hardiness. The Oldenburger division of the black and white lowland cattle showed an earlier rise to the peak of production and a quicker decline afterwards, which is attributed to the influence of some Shorthorn blood introduced long ago. The Angler breed showed less extreme changes than the black and white lowland cattle. These differences

Year on test	Milk quantity		Fat (per cent)	Total fat	
	Kg.	Relative		Kg.	Relative
2nd	4111	76.3	3.57	146.7	77.0
3rd	4632	85.8	3.59	166.1	87.2
4th	5082	94.3	3.57	181.5	95.3
5th	5268	97.8	3.57	188.0	98.7
6th	5373	99.7	3.55	190.5	100.0
7th	5388	100.0	3.53	190.0	99.7
8th	5296	98.2	3.54	187.6	98.5
9th	5212	96.2	3.51	182.8	96.0
10th	5109	94.8	3.52	179.8	94.4
11th	5049	93.7	3.48	175.5	92.1
12th	5150	95.6	3.38	174.3	91.5

were slight and for practical purposes it may almost be said that there were no breed differences in the relative changes of production with age.

The change in fat percentage with advancing age was small and no breed differences were found in the shape of that curve. The maximum production of total fat was reached in the sixth association year which would be at an age of about 8 or 9 years. The following table shows the averages by year on test. The year the cow first came on test was omitted, since that would be fragmentary in nearly all cases.

J.L.L.

BUTTER

670. The Yeast and Mold Service in Relation to Quality Improvement.

W. H. SPROULE, Dairy Dept., Ontario Agr. College, Guelph, Ont.
Canad. Dairy and Ice Cream Jour., 20, No. 1: 50. 1941.

Yeast and mold counts have been recognized for a considerable period of time as playing an important part in laboratory control of butter quality. While butter containing numerous yeasts and molds might give good commercial satisfaction at times, as shown by some of the work accomplished, nevertheless, the larger creameries recognized that butter with a low yeast and mold content was a better risk for storage purposes than butter made in a less sanitary way. In reviewing the progress made during 8 years, D. B. Shutt reported marked improvement as the work progressed. In 1928, 34.8 per cent of submitted samples had counts of 10 yeasts, or less, per cc. as compared with only 1.2 per cent in 1921. The mold counts also showed improvement; 61.8 per cent of the samples showed counts of 10 or less in 1921, whereas, in 1928, 89.9 per cent fell in this class. The butter laboratory is at present making analyses for yeast and mold on samples submitted by creameries which have applied for the service. O.F.G.

671. The Experimental Error in the Plate Count Examination of Butter.

E. G. PONT, Dept. of Agriculture, Sydney, Australia. Jour. Dairy Res. 12, No. 1: 24-34. 1941.

In an investigation into the experimental error of the plate count of butter, 154 boxes of butter were examined by plating in triplicate, in a dilution of 1/500, each of three 1-gram samples per box. The means of the triplicate counts on any one sample, judged by conformity to the Poisson distribution, were considered to give reasonably satisfactory estimates of the bacterial content of the samples. The between-sample variability was shown by transforming the counts to logarithms and calculating the coefficient of variation in respect of each box. In the distribution of the coefficients approximately 50 per cent was found to lie on either side of a 4 per cent level, while 10 per cent gave values higher than 14 per cent.

In a further study 12 boxes of butter were selected for quality and uni-

formity and data were secured from the examination of 7 1-ounce samples selected at random from each box. Using the method of analysis of variance, the results indicate that the estimates of within sample variance obtained would be regarded as estimates of a common variance. High significant differences were found, however, among the between-sample mean squares and the variability was found to be excessive in six of the twelve boxes examined.

The author points out that owing to the excessive between-sample variance found, the result of a single plating used as an index of the mean bacterial population of a box of butter may be quite inaccurate. It is only when a high estimate is encountered (*e.g.*, several hundred thousand or more per gram) that any real degree of significance can be attached to the result. Errors arising from technique would be unlikely to influence a normally low count to this extent. The occurrence of butter giving rise to such a count, even though it appeared only in parts of a box or a churning, would, from the standpoint of quality control, indicate the need for remedial measures.

S.T.C.

672. Facts to Know about Packaging Butter. L. C. THOMSEN. Amer. Butter Rev., 2, No. 4: 114, 116, 128. 1940.

Figures from several localities in this country show surprisingly large quantities of bulk butter are still sold to the public. Most of this comes to the dealer in spruce tubs, notorious for their tendency to transmit wood flavor to butter. Paraffin retards, but does not prevent, absorption of these flavors. Anti-oxidants applied to parchment liners, according to preliminary work, do not prevent absorption. The casein-formalin treatment, while perhaps effective, has not been looked upon favorably in this country. Avoidance of air pockets in packing butter reduces aerobic conditions necessary for the growth of bacteria causing spoilage; careless storage conditions for sterile parchment may result in mold and yeast contamination; and treatment of parchment circles and liners with salt brine and calcium propionate further reduces mold. The use of the latter alone in 10 per cent solution has been reported as responsible for surface mottling of butter.

The author predicts as the next great advance in packaging butter, a continuous churn with packaging following directly from the churn. Wholesaler's demands for amount of overweight per 64 pound tub, he states, varies from 6 to 12 ounces. From two to fifteen per cent of butter reaches the market underweight. On the average when butter is cut the overweight per uncartoned pound print is $\frac{1}{4}$ ounce. Losses in cutting and in overweight may amount to $\frac{3}{4}$ to one pound per 64 pound tub. Overweights given per pound of butter amounts to $\frac{3}{8}$ pounds per hundredweight of butter cut. Shrinkage of pound prints of dry wrapped butter is about $\frac{1}{16}$ ounce more than for prints wet wrapped. Machine wrapping and cartoning costs for

one pound prints vary from 0.76 to 1.35 cents per pound; and for quarter pounds, from 1.0 to 1.36 cents per pound. The latter, uncartoned, costs 0.75 cents per pound. P.S.L.

673. Manufacture and Use of Butter Culture. N. E. FABRICIUS. Amer. Butter Rev., 2, No. 3: 74, 92. 1940.

The author reviews the arguments for and against the use of starter, the organisms responsible for flavor in starter, the products formed by starter bacteria, and the problems involved in the successful carrying on of cultures. He recommends heavy inoculation of cultures, one pint or quart per 10 gallons of milk; the addition of two ounces of citric acid crystals dissolved in $\frac{1}{4}$ pint of hot water per 10 gallons of milk; ripening the culture to a higher degree of acidity than ordinarily practiced, and using taste or the creatine test rather than acidity test for determining the degree of ripeness; quick cooling with vigorous stirring; and cooling to a low temperature to prevent reducing diacetyl to flavorless, 2, 3-butylene glycol. As between addition of starter to cream at 70° F. allowing to grow a few hours and cooling, or addition of starter to cooled cream and holding 8 to 12 hours before churning, the author prefers the latter procedure because a greater quantity of the acetylmethyl-carbinol is oxidated to diacetyl. P.S.L.

674. Testing Cream for Mold Mycelia. C. H. PARSONS. Amer. Butter Rev., 2, No. 11: 382-384. 1940.

The details of procedure for the Parsons Visual Mold Test together with a list of required equipment is given in this article. The author suggests classification of cream into four grades according to mold content. Being both inexpensive and simple the test is recommended as a routine measure in cream stations and butter plants. P.S.L.

675. On the Receiving Line. V. SCHWARTZKOPF. Amer. Butter Rev., 2, No. 12: 406, 408, 410, 412. 1940.

Quality of cream will be maintained if it is handled promptly at the plant, graded and churned by grade, contamination avoided, metallic taint prevented through use of well-tinned cans, and return to the farm of clean, dry cans. For cleaning cans the use of soft water will prevent reduction of washing machine efficiency by preventing formation of sludge which clogs pipes, tank and pumps. Washing powder that produces no bacteria harboring sludge is best. It must be free rinsing and capable of removing all deposits quickly and completely and be a good water softener if hard water is used. Temperature of water should be 140°-145° F. and alkalinity, 0.3 per cent or less. After washing, the can should be rinsed in clean hot water at 190° to 200° F., although the rinse, if hard water, leaves a film of mineral

on the can. Use of softened water eliminates this difficulty. After steam or hot water sanitization it is desirable to dry the can with air heated to 250° F. or higher. Such procedure seldom gives an absolutely sterile can. Sterilizers such as 1:100 Zephrein are efficient but costly. Sterilization by light gives little promise. Straight-side cans are more easily cleaned.

P.S.L.

676. **Counting Mold Mycelia in Butter.** G. W. SHATTUCK, JR. Amer. Butter Rev., 3, No. 1: 10, 12. 1941.

A complete outline of the method for counting mold mycelia is given. Recommendation is made that the microscope be standardized to cover a field 1.382 mm. in area and that no field is reported positive unless one filament or the combined length of the two longest filaments exceeds $\frac{1}{4}$ the diameter of the field.

Filters aid in picking out the filaments. Use of five per cent aqueous solution of crystal violet as a stain in preparing the sample outlines the mycelia sharply making possible their identification as single or broken filaments and making their measurement more conclusive. Efforts to simplify mold mycelia counting procedure have been disappointing. In case the count is low or high, the counting of 50 fields is satisfactory, but where the count is close to 60 per cent positive, the examination of 100 fields is preferable. A tolerance of 10 per cent difference in counting between technicians is allowable. Observation leads to the theory that length of the mold filament is correlated with age of cream.

P.S.L.

677. **Cream Grading and the Future of the Butter Industry.** C. E. LACKNER, Dept. of Agr., Toronto, Ont. Canad. Dairy and Ice Cream Jour., 20, No. 2: 62. 1941.

The author points out that, although the fear of surplus Canadian butter is passed now, there is likely to be a surplus again when world conditions become normal. The quality of Ontario cheese has been good but a considerable portion of the butter has been of low grade due primarily to poor quality in the cream. Looking ahead, therefore, to normal market conditions when a surplus of butter can be expected it is essential that a sound cream-grading program be promulgated now.

O.F.G.

678. **Bacteria in Well-Waters.** C. H. CASTELL AND E. H. GARRARD, Ontario Agricultural College, Guelph, Ont. Canad. Dairy and Ice Cream Jour., 20, No. 3: 18. 1941.

As pasteurization of cream and improved sanitation has decreased certain types of butter and cream spoilage, the importance of trouble from what appears to be minor sources has become more significant. One of the most important of these is water used by creameries and owing to the peculiar

characteristics of water bacteria, the milder and less salty butter is made, the more their activity will be noticed. Ten per cent of the samples examined were found unfit for human consumption, 30 per cent showed the presence of butyric acid forming anaerobic bacteria, a majority of the waters contained organisms which were oxidase-positive, and approximately 85 per cent contained organisms capable of growing at a temperature within 4 or 5 degrees of freezing and at the same time capable of decomposing fat and curd. Results suggested the presence of *Pseudomonas fragi* and *Pseudomonas fluorescens*.
O.F.G.

679. Factors Influencing Mold Mycelia in Cream. P. R. ELLIKER, Purdue Univ. Natl. Butter and Cheese Jour., 32, No. 7: 8. 1941.

The velvety, white growth commonly found on the surface of sour milk or cream is *Oospora lactis*, "milk mold." It gets into milk and, eventually, cream and butter through dust, dirt, manure and utensils. Some strains grow as low as 40° F., others as high as 100° F., but ideal temperatures approximate 75° F. It is destroyed by proper pasteurization. Growth of *Oospora lactis* is favored by a slightly acid reaction and is retarded or inhibited by high acidity, lack of air, presence of salt and possibly propionic acid or its salts. Its presence in butter with yeasts and other molds indicates unclean churns or equipment. It forms part of the surface flora of some cheese. Delivery of cream 3 times weekly eliminates the mold problem if clean utensils and separators are used and if cream is stored at 60° or lower. The mold content of gathered cream decreases as the fat increases from 30 to 50 per cent, probably because it is machine- rather than hand-separated but perhaps because rich cream may be a less favorable medium for mold growth. Frequent stirring of cream decreases the mold count on butter but may injure the quality of the butter because it encourages undesirable changes in the cream. A large surface area of cream in relation to amount of cream increases mold growth. Delays in neutralizing and pasteurizing cream permit mold growth. During the processing of cream and butter, mold filaments are broken. Although some of the mold may go into the buttermilk, still a high mold content in the cream causes a high mold content in butter.
W.V.P.

680. Safeguarding Butter Quality. B. F. McKIBBEN. Amer. Butter Rev., 2, No. 3: 78, 92. 1940.

In a discussion of the thirty-five off flavors listed in the government score card for butter this writer emphasized the danger of transmitting lubricating oil flavors to cream from oil lubricated pumps, and cites the work of Turgasen as to the frequency of cheesy flavors from contaminated water.

P.S.L.

681. Recent Trends in Neutralization. LEE H. MINOR. Amer. Butter Rev., 3, No. 3: 90, 92. 1941.

Calcium in lime neutralizers has a greater affinity for casein or curd than for lactic acid and tends to increase viscosity of cream, produce lime flavor, and clog filters; sodium in neutralizers, while excellent for neutralizing lactic acid, has a tendency to create foam and has been accused as being responsible for soapy flavor. Combination of the two lessens the effect of each. With the vacuum process of pasteurization neutralizer may be added to the cold cream in the forewarmer, allowing it to act on the acid before pasteurization temperature is reached, reducing foaming and danger of saponification of fat. Dilution of the neutralizer with water is important, the amount increasing with the causticity of the alkali. P.S.L.

CHEESE

682. "Phage" in Cheesemaking. C. K. JOHNS, Div. Bacteriology and Dairy Res., Science Service, Ottawa, Ont. Canad. Dairy and Ice Cream Jour., 20, No. 2: 18. 1941.

Most makers of Cheddar cheese have had experience with slow acid development, an occurrence which is generally accepted as one of those things that cannot be explained and about which nothing can be done except to get a new starter. In New Zealand considerable use is made of single strain lactic acid bacteria starters. When such a starter becomes contaminated with bacteriophage the bacteria are destroyed practically 100 per cent and acid development ceases abruptly. In Canada, on the other hand, the starter is usually a mixture of several different strains or species of desirable bacteria. Since a phage generally attacks only one strain, or a few closely related strains, the result is usually a slow development of acid. The author, however, found a phage different from that reported in New Zealand which attacked every one of 10 different organisms found in the starter in use. Several outbreaks of slow acid development were found each of which disappeared when the plant and equipment had received a thorough house-cleaning and sterilizing treatment. O.F.G.

683. The Drying of Cheese Whey and of Acid Casein Whey by the Roller Process. R. WAITE, The Hannah Dairy Res. Inst., Kirkhill, Ayr. Jour. Dairy Res., 12, No. 1: 71-77. 1941.

Sodium or potassium compounds were found to be unsuitable for neutralizing cheese whey prior to drying. Neutralization with calcium hydroxide gave a satisfactory product. Reduction of the acidity of hydrochloric acid casein whey to 0.18 per cent by the addition of calcium hydroxide allowed satisfactory neutralization. S.T.C.

684. Starter Cultures for Cheese Manufacture. Further Attempts to Eliminate Failures Due to Bacteriophage. H. R. WHITEHEAD AND G. J. E. HUNTER, Dairy Res. Inst. (N.Z.), Palmerston North, New Zealand. Jour. Dairy Res., 12, No. 1: 63-70. 1941.

Bacteriophages for lactic streptococci were found to occur in the atmosphere of commercial cheese factories. This was established in three ways: (a) aspiration of air, (b) exposure of sterilized skim milk, and (c) exposure of inoculated agar surface. Finely divided particles of whey emitted from the whey separator appeared to be the main vehicle for the air-borne phage although whey contaminated dust probably also played a part. Protection of the starter from air-borne phage eliminated phage failures. A separate building for starter propagation is suggested as the only means of insuring this at present.

S.T.C.

685. The Consistency of Cheese Curd at the Pitching Point and Its Bearing on the Firmness and Quality of the Finished Cheese. G. W. SCOTT BLAIR AND F. M. V. COPPEN, Natl. Inst. Res. in Dairying, Univ. Reading, Reading, Eng. Jour. Dairy Res., 12, No. 1: 44-54. 1941.

Further studies are reported on the use of the method previously described (JOUR. DAIRY SCI., 24, No. 2: A26. 1941) for measuring the consistency of cheese curd at the "pitching" point. The value determined W/h was found to be an excellent criterion of the properties of the cheese curd assessed by an expert cheese maker.

No significant relationship was found to exist between the firmness of the curd at "pitching" and acidity. The most usual values (medians) for "pitching" consistency were compared for 4 different factories and it was shown that differences in technique may be associated with the same "pitching" consistency and produce very similar cheese, but that in other cases good cheese may be produced from very different "pitching" consistencies.

S.T.C.

686. "Slowness" in Cheesemaking. J. HARRISON AND D. V. DEARDEN, Natl. Inst. Res. in Dairying, Univ. Reading, Reading, Eng. Jour. Dairy Res., 12, No. 1: 35-43. 1941.

Two cases of "slowness" in cheesemaking are reported in which the trouble was eliminated by changing the source of the starter. The principal cause of the "slowness" studied appears to have been the inability of the streptococci in the starter cultures used to grow at scalding (cooking) temperatures. The necessity of using a starter capable of normal growth at 40° C. (104° F.) is thus demonstrated.

Attempts to isolate either bacteriophage or "non-acid" organisms failed.

S.T.C.

687. The Problem of Rancidity in Cheddar Cheese. E. G. HOOD, I. HLYNKA AND C. A. GIBSON, Dept. of Agr., Ottawa, Ont. Canad. Dairy and Ice Cream Jour., 20, No. 3: 26. 1941.

The odor and flavor of rancid cheese are characteristic of those of butyric acid. It has been shown experimentally that it is possible to produce rancidity by the addition of butyric acid to the cheese milk. Lipase gives rise to butyric and other fatty acids when added to cheese milk. Raw milk contains lipase. Free butyric acid may be produced from casein, lactose, glycerol or butterfat. The most likely cause of rancidity is the action of lipase on butterfat to produce free butyric acid. In an experimental study of the problem it was found:

1. Typical rancid cheese were reproduced with the addition of lipase or homogenized milk to the cheese milk. Homogenization activates the naturally present lipase.

2. Rennet and pepsin partially inactivated additions to cheese milk of lipase or homogenized milk.

3. A number of the cheese made from inactivated milk fell in grade after a storage period of 6 weeks.

4. A relation between unclean, dirty, etc., flavors and rancidity in cheese was suggested.

O.F.G.

688. Some Micro-organisms Associated with Gassy Swiss Cheese. HARRY H. WEISER, Ohio State Univ., Columbus, Ohio. Natl. Butter and Cheese Jour., 32, No. 7: 20. 1941.

Splitting rinds were observed on the edge and extending 4 to 5 inches toward the center of the Swiss cheese. The defect was accompanied by gas formation, lack of characteristic eye formation, poor body, bitter flavor, sometimes yeasty odor and lack of Swiss flavor. Bacteriological examination disclosed that associated with the occurrence of this defect were considerable numbers of lactose fermenting yeasts and anaerobic or facultative anaerobic bacteria of the *Clostridium perfringens* group. Other organisms may be involved. Practical control lies in the use of good milk and good active starter.

W.V.P.

689. Pasteurization for Cheesemaking. G. S. BIRBY, Cherry-Burrell Corp., Chicago, Ill. Natl. Butter and Cheese Jour., 32, No. 7: 14. 1941.

Three types of pasteurizers commonly used are: the internal tube heater with tubular surface regenerator; a flash pasteurizer with a tubular surface type regenerator; and the plate pasteurizer. All are of the "flash" type and use maximum temperatures of 160°-165° F. frequently with a 16 to 20 second hold for increased bacterial destruction. Costs of pasteurizing

100 lbs. of milk approximate 1.5 cents for steam and 0.35 cents for power. Two to four man hours daily are required for cleaning. Extra costs are offset by benefits of increased yield, uniformity and quality. Health authorities, eventually, will demand that all cheese milk be pasteurized as Kentucky does now. W.V.P.

690. Survey of Cheese Preferences. EDITORIAL. Amer. Butter Rev., 3, No. 5: 162, 196. May, 1941.

This editorial presents results secured in a survey representing 3.5 per cent of the families in Milwaukee, Wisconsin, as to their cheese preferences. Families purchasing packaged cheese in 1937 were 59.9 per cent and in 1940, 56.1 per cent, the numbers being 112,701 in 1937, and 110,569 in 1940. Of package cheese users, 48.8 per cent preferred the 8-ounce package, 26.5 per cent a smaller size, 12.4 per cent a 2-ounce carton, and 12.3 per cent preferred a one-pound package. In 1937, 82.6 per cent of the population surveyed regularly used bulk or loaf cheese; in 1940 the number had increased to 92.9 per cent. Of bulk cheese purchased 64.2 per cent was American type of cheddar, 22.9 per cent brick, and 12.9 per cent Swiss. Average consumption of bulk cheese per family has shown a variation of only one-tenth of a pound since 1934, monthly consumption per family being 2.1 pounds.

P.S.L.

691. Manufacture of Acid-rennet Type Cottage Cheese. D. W. GLOVER AND L. H. BURGWARD, Ohio State Univ., Columbus, Ohio. Milk Dealer, 30, No. 8: 42-50. May, 1941.

Directions are given for the manufacture of acid-rennet type cottage cheese. Directions for using homogenized milk returns are included. The authors draw the following conclusions:

1. Start with sweet, clean skim milk and pasteurize it by heating to 143° F. for 30 minutes. Higher pasteurizing temperatures may be detrimental to the texture of the resulting curd.
2. Temper the milk to 70° F. if the long set is to be used or to 85° if the short set is to be employed.
3. Add starter at the rate of 0.5 per cent of the weight of the milk used for the long set or 3.0 per cent to 5.0 per cent for the short set. A choice between the long and short set methods will depend upon the work schedule of the particular plant.
4. Add rennet at the rate of 1.0 cc. per 1,000 pounds of milk in the vat.
5. Utmost care should be exercised in taking the whey sample for the acidity test to insure that no curd particles are present. The use of the whey well affords a satisfactory method of obtaining the sample.
6. Cut the curd when the acidity of the whey reaches 0.52 per cent.

7. The cooking may be accomplished by the use of hot water in the jacket of the vat and by adding hot water directly to the curd. The use of water in the cooking process enhances the firming of the curd, thus saving time in the cooking process.

8. The use of tempered wash waters prevents matting and the breaking up of curd made brittle by too sudden cooling.

9. The curd should be thoroughly chilled before creaming and packaging. Unchilled curd has a tendency to be tender and is prone to break up in the creaming process.

10. The use of homogenized milk results in a product with superior quality, and at the same time provides a method for utilizing returns.

C.J.B.

CHEMISTRY

692. Analysis of Proteins. 13. Caseo-Phosphopeptone. J. LOWNDES, T. J. REW MACARA AND R. H. A. PLIMMER, St. Thomas's Hospital Medical School, London, S. E. *Biochem. Jour.*, 35, No. 3: 315-319. March, 1941.

Judging from its N and amino-N contents, caseo-phosphopeptone is an octapeptide containing two H_3PO_4 groups, 2 mol. glutamic acid and 2 mol. serine. Its acidity indicates the presence of another mol. of a dicarboxylic acid, leaving 3 mol. of simple amino-acids.

V.C.S.

693. Methods of Measuring the Rate and Extent of Oxidation of Fats. FRANK C. VIBRANS, Amer. Meat Institute, 59 East Van Buren St., Chicago. *Oil and Soap*, 18, No. 5: 109. May, 1941.

This paper discusses the following tests and methods for measuring the rate and extent of fat oxidation: 1. Kreis test; 2. Issoglio-Kerr test; 3. Aeration methods; 4. Photochemical methods; 5. Oxygen absorption; 6. Oxygen absorption-Peroxide method.

V.C.S.

694. A Convenient and Efficient Method for the Determination of the Digestibility of Fats with Pancreatic or Other Lipases. J. R. KOCH AND SISTER M. DOLOROSA DUELLMAN, Marquette Univ., Milwaukee, Wis. *Oil and Soap*, 18, No. 4: 86. Apr., 1941.

A convenient and easy to operate method for carrying out hydrolysis experiments with pancreatic lipase is described. The authors list the following advantages for this method:

1. It allows five determinations to be carried out at one time.
2. It shows a smooth course of reaction in every case.
3. It gives more complete hydrolysis since the acids are used up as formed.

4. It keeps the pH on the alkaline side under constant control and in the range where the enzyme is most active.

5. It eliminates the removal of aliquots and the killing of the enzyme.

6. It makes it possible to make determinations directly in the digestion mixture.

V.C.S.

695. Antioxidants for Edible Fats and Oils. H. S. OLCOTT, Mellon Institute, Pittsburgh, Pa. *Oil and Soap*, 18, No. 4: 77. Apr., 1941.

Ascorbic acid, vitamin C, possesses antioxidant activity of the acid type while the tocopherols, that is vitamin E, possess the properties assigned to the phenolic inhibitors. Theoretically, combinations of the two should be particularly advantageous antioxidants, and actually the data confirm this assumption.

Purified lecithin possesses no antioxidant activity but with the commercial product the cephalin fraction carries the inhibitor action.

Cereal flours and particularly oat flour possess antioxidant properties. Phospholipids may account for part but not all of the effective principle of oat flour.

Cottonseed meal is an excellent antioxidant in fats and oils.

V.C.S.

696. The Mechanism of the Autoxidation of Fats. H. A. MATTILL, State University of Iowa, Iowa City, Iowa. *Oil and Soap*, 18, No. 4: 73. Apr., 1941.

Although the chemical changes during the induction period are very different and less obvious than those that follow it, they are, from a practical point of view, more important because once the induction period is past the damage is done. During oxidation the fats pass through two stages: a latent or induction period of variable length during which the amount of oxygen absorbed is small, followed by a period of rapidly accelerating oxygen absorption. The end of the induction period usually coincides with or immediately precedes the first appearance of the products of organoleptic rancidity.

Numerous tests have been devised for detecting the degree of susceptibility of a fat toward oxidation. These tests are based upon the estimation of some chemical change, yet the order of reason for the reaction is not clearly understood.

This paper deals with some of these chemical changes. No all-inclusive theory of autoxidation can yet be formulated.

V.C.S.

697. A Convenient Method for the Rapid Estimation of Carotene in Butterfat. WILLIS D. GALLUP AND A. H. KUHLMAN, Oklahoma Agr. Expt. Sta., Stillwater, Okla. *Oil and Soap*, 18, No. 4: 71. Apr., 1941.

A simple method is described for determining the carotene content of

butterfat under conditions where extreme accuracy is not required. A direct comparison is made of the color of the melted fat with that of known concentrations of potassium dichromate solution.

The dichromate solutions are prepared by dilution of measured amounts of a 0.2 per cent stock solution to a volume of 25 ml. These dilute solutions and the fat samples are contained in cylindrical sample bottles of uniform diameters and their color matched with the aid of a comparator block placed before a "daylight" lamp.

The authors give a table showing the carotene content of butterfat in micrograms per gram corresponding to the color produced by various concentrations of potassium dichromate. The carotene content may be calculated from the formula $X = \frac{Y - b}{a}$ in which X is the micrograms of carotene per gram of fat, Y is the concentration of the matching dichromate solution in per cent, and b and a are factors, 0.012 and 0.018, respectively.

V.C.S.

698. The Melting Points of Binary Mixtures of Oleic, Linoleic, and Linolenic Acids. H. W. STEWART AND D. H. WHEELER. Oil and Soap, 18, No. 4: 69. Apr., 1941.

The oleic-linoleic acid system has eutectics for the alpha and beta forms of oleic acid of 75.2 and 76.3 mole per cent linoleic acid, at -10.0° and -9.8° , respectively.

Linoleic and linolenic acid mixtures show only melting points intermediate between the pure acids.

The oleic-linolenic acid system has eutectics for the alpha and beta forms of oleic acid of 82.7 and 85.5 mole per cent linolenic acid, at -15.7° and 15.1° , respectively.

V.C.S.

699. Enzymic Proteolysis. 4. Amino-Acids of Casein Phosphopeptone. M. DAMODARAN AND B. V. RAMACHANDRAN, Univ. Biochem. Lab., Chepank, Madras. Biochem. Jour., 35, Nos. 1 and 2: 122-133. Jan., 1941.

By digestion of "paranuclein" from casein with trypsin an enzyme-resistant phosphopeptone of constant composition had been isolated in the form of its barium salt.

The phosphopeptone was shown to contain 10 amino-acid units, viz., 3 mol. glutamic acid, 3 mol. of isoleucine and 4 mol. of serine. The absence of other hydroxy- or dicarboxylic-amino-acids has been demonstrated by indirect methods.

A method is described for the approximate estimation of serine in the absence of other hydroxyamino acids.

V.C.S.

700. The Component Acids of Phosphatides Present in Cow's Milk Fat.

THOMAS PERCY HILDITCH AND LIONEL MADDISON, Dept. Indust. Chem., Univ. Liverpool. Biochem. Jour., 35, Nos. 1 and 2: 24-30. Jan., 1941.

The typical milk fat glyceride acids of low molecular weight are wholly absent from the phosphatide acids. The component fatty acids of milk fat phosphatides have little in common with those of the milk fat glycerides; on the other hand, they bear more general similarity to those of the phosphatides of the ox liver.

The authors report the following acids and amounts in the phosphatides separated from Swiss and English butters:

Acid	Per cent by weight	
	"Swiss"	"English"
Myristic	3.2	5.5
Palmitic	21.0	13.4
Stearic	7.3	9.0
As Arachidic	12.3	20.9
As $C_{26}H_{52}O_2$	5.2	10.0
Hexadecenoic	4.3	4.9
Oleic	32.5	23.5
As Octadecadienoic	6.4
As C_{20-22} unsaturated	7.8	12.8

V.C.S.

701. Preliminary Experiments on the Vapor Pressure of Dairy Products.

G. W. SCOTT BLAIR, F. J. DIX AND A. WAGSTAFF, Natl. Inst. Res., in Dairying, Univ. Reading, Reading. Eng. Jour. Dairy Res., 12, No. 1: 55-62. 1941.

The vapor pressure was determined as follows: A large number of air-tight tobacco tins of diameter about 4.5 in. were fitted with a simple wire clip to hold a pad of cotton wool about 2 in. square, against the lower side of the lid. The cheese were spread thinly on the bottom of the weighed tin, which was then reweighed as quickly as possible to avoid evaporation. A pad of cotton wool was then soaked in a salt solution of known concentration and vapor pressure and was lightly pressed out to prevent dripping. The pad was quickly clipped against the lid and the tin tightly closed. After 48 hours at a constant temperature, approximately 60° F. (15.6° C.), the lid was removed and each tin and cheese reweighed immediately. The change in weight per gram of cheese was then plotted against salt concentration and that concentration corresponding to no change in weight was obtained by interpolation.

Vapor pressure moisture curves are given for a number of different varieties of cheese. The vapor curve was found to be influenced by the

amount of salt in the cheese, but differences between varieties cannot be accounted for entirely in terms of differences in salt content.

A preliminary experiment on the relationship between vapor pressure of Stilton cheese and amount of blueing indicated that such a relationship does in fact exist, but that a much larger experiment is required before the connection is fully understood.

Preliminary experiments on the measurement of the vapor pressure of milk showed that additions of 2-3 per cent of water in milk can be detected.

S.T.C.

702. **Electrical Testing Device.** NATHAN SCHNOLL. *Amer. Milk Rev.*, 3, No. 5: 106, 107. May, 1941.

As a method for checking the percentage of free caustic in a washing machine the solubridge is a modified Wheatstone bridge for measuring conductivity of caustic solutions. By using a rotary switch different tanks of caustic solutions may be checked with the same bridge. Used with a total alkalinity test it gives positive knowledge of the strength of the washing solution.

P.S.L.

703. **Autoxidation Measurements on Fatty Oils Using Barcroft-Warburg Apparatus.** W. R. JOHNSON AND CHARLES N. FREY, Fleischmann Labs., Standard Brands, Inc., New York, N. Y. *Jour. Indus. and Engin. Chem., Analyt. Ed.*, 13, No. 7: 479-481. 1941.

The Barcroft-Warburg equipment was used to measure induction periods of sesame and cottonseed oils at temperatures from 50° C. to 100° C., most of the determinations being made at 100° C. in an atmosphere of oxygen. The data show that the Barcroft-Warburg technique can be extended to elevated temperatures with convenience and precision.

B.H.W.

704. **Determination of Thiamin by the Thiochrome Reaction.** R. T. CONNOR AND G. J. STRAUB, Central Labs., General Foods Corp., Hoboken, N. J. *Jour. Indus. and Engin. Chem., Analyt., Ed.*, 13, No. 6: 380-384. 1941.

This method for the determination of thiamin first proposed by Jansen is based on the measurement of the florescence produced by thiochrome formed by the oxidation of thiamin with potassium ferricyanide in an alkaline solution. This paper defines more exactly than has been done previously, the optimal conditions for carrying out the thiochrome procedure and suggests some improvement in the equipment used. Extraction and hydrolysis of the sample are carried out in the same vessel and for the enzymatic hydrolysis of cocarboxylase, the enzyme clarase is introduced. Optimal conditions for the oxidation of thiamin and for the extraction of the thio-

chrome formed are given. The method is in close agreement with biological assays and has been applied to various types of natural products including whey and skim milk powders. B.H.W.

705. Combined Determination of Riboflavin and Thiamin in Food Products. R. T. CONNOR AND G. J. STRAUB, Central Labs., General Foods Corp., Hoboken, N. J. *Indus. and Engin. Chem., Analyt. Ed.*, 13, No. 6: 385-388. 1941.

A rapid and accurate procedure is described which makes possible the determination of both vitamins on the same sample. The method is an extension of the one proposed for thiamin and gives results closely agreeing with biological assays. It has been applied to grains, dairy products, fresh and frozen vegetables. Rapid destruction of riboflavin in aqueous solutions by light was found. Destruction by diffused light of the laboratory occurred irrespective of pH but in artificial light destruction was slower and dependent upon pH. The pH range from 2 to 8 was studied. Ferree's procedure for adsorption of riboflavin on Supersorb was modified to use a smaller extraction column and a study was made of Corning glass filters suitable for the fluorometric determination of riboflavin. B.H.W.

706. Distribution of Nitrogen and Protein Amino Acids in Human and Cow's Milk. ELIOT F. BEACH, SAMUEL S. BERNSTEIN, OLIVE D. HOFFMAN, D. MAXWELL TEAGUE AND ICIE, G. MACY, Res. Lab. Children's Fund of Michigan, Detroit. *Jour. Biol. Chem.*, 139, No. 1: 57. May, 1941.

The amounts (in milligrams) of seven amino acids contained in the proteins of 100 ml. of human and cow's milk were calculated to be as follows:

	Cow's milk	Human milk
Histidine	59	12
Arginine	127	40
Lysine	223	50
Tyrosine	197	50
Tryptophane	43	19
Cystine	23	20
Methionine	104	18

In the proteins of cow's milk the preponderance of sulphur is in the form of methionine, with very little in the form of cystine, while in the proteins of human milk the sulphur is about equally divided between cystine and methionine. V.C.S.

DISEASE

707. Lancefield Group B Streptococci (Str. agalactiae) on the Hands of Milkers and Others. J. HARRISON, *Natl. Inst. Res. in Dairying*,

Univ. Reading, Reading, Eng. Jour. Dairy Res., 12, No. 1: 18-23. 1941.

Lancefield group B streptococci were recovered from the hands of all but one of the milkers (eight in all) on three different farms. A routine method of disinfecting the milkers' hands consisting of a soap and water wash followed by a rinse in sodium hypochlorite solution containing about 800 parts per million available chlorine was used on the farms. Following, in most instances, the routine cleaning, the hands were scrubbed with a hard nail brush in sterile milk and the milk examined for the group B streptococci using Edwards medium.

Since the organisms recovered from the hands of the milkers had the biochemical reaction of *Str. agalactiae*, and since none was recovered from the hands of non-milkers, the milkers' hands were considered to be a potential source of infection to cows. S.T.C.

708. Die Agglutinationsmethode nach Stableforth und Willems zur Feststellung der Rinderbruzellose. (The Agglutination Test of Stableforth and Willems in the Diagnosis of Brucellosis in Cattle. R. ENDRESS, (Aus der vet.-med. Abteilung d. Reichsgesundheitsamtes, Zweigstätte Dahlem.) Ztschr. f. Infektionskrank., Parasitäre Krank. u. Hyg. der Haustiere, 56, No. 4: 297-320. 1940.

Since the agglutination test is the most important and widely used test for the diagnosis of this disease, attempts have been made to standardize it for international use. A standard test is necessary because of the great variation in methods used by different workers. Stableforth in London, and Willems in Brussels, have suggested standard methods for making the tests and this investigation was an attempt to evaluate them.

This writer used five strains of *B. bovis*. The cultures were grown on 2 per cent glycerine agar, pH 7.5, for 3-4 days at 37° C. The suspension was made in phenolized salt solution, filtered through gauze, and heated at 70° C. to kill the organisms. It was examined culturally and microscopically for purity. This suspension was standardized by a Leitz photometer to the density recommended by Stableforth (Brown No. 4 = 97 cc. 1 per cent H₂SO₄ plus 3 cc. 1 per cent BaCl₂ solution). The suspension was placed in the refrigerator 2-3 weeks at 5-6° C. and the agglutinability tested with a known serum. Stableforth kept a dried serum to which was added sterile saline at time of use to give dilutions of 1:25 to 1:750 in 30 tubes. To the various serum dilutions was added an equal volume of the antigen, thus doubling the dilution. Appropriate controls were included. The tests were incubated at 37° C. for 24 hours and for one hour at room temperature.

As a guide in the evaluation of the antigen, Willems' modification of the formula of Stableforth was used. This consists of determining the agglu-

tion constant and the agglutinability index. In the agglutinability index the titer limit of a suspension of the antigen and a standard serum is observed. The agglutination constant is the relation between the diagnostic titer of the infection and the agglutinability index. According to Willems the former is 50 and the agglutinability index for his particular antigen was 700; therefore, the agglutination constant was $50/700 = 0.07142$. With the aid of a standard serum the agglutinability index of other antigens can be determined. By multiplying the numbers thus obtained by the agglutination constant the infection titer can be determined. The agglutinability index for the English strains of the organism studied was 500, for the German strains 550. The infection titer for the latter strain was $550 \times 0.07142 = 39.3 = 40$. A 25 per cent agglutination in a 1:40 dilution was considered as a positive reaction.

The sensitivity of all cultures used in preparing the antigens was tested before they were used. In routine testing, dilutions of the serum are made by using 1 cc. of serum and 4 cc. of 0.5 per cent salt solution. This is diluted to 1:80 or above. In this investigation 1:40 was considered as the infection titer.

From this study it was concluded that the agglutination method of Stableforth and Willems for the diagnosis of brucellosis in cattle is useful and reliable.

The advantage of the method lies in the accuracy and greater ease of determining the results. For the diagnosis in doubtful cases the method has an advantage.

The utilization of several strains of bacteria in preparing the antigen was considered necessary in order to make it as active as possible.

The difference obtained by the use of the Stableforth-Willems method and those methods now commonly used in Germany and the complement fixation test are negligible.

L.D.B.

709. **The Role of Milk in Tuberculosis.** H. A. REISMAN, Queens General Hospital, Jamaica, N. Y. *Certified Milk*, 16, No. 179: 5. March, 1941.

Milk not only plays an important role as the vehicle through which this disease is transmitted to man, but it also plays an important role in the treatment of infected individuals, because of the availability of its calcium. The author concludes that the bovine strains of tuberculosis in man can be completely eliminated in two ways:

(1) The eradication of the disease at its origin by tuberculin testing, and the slaughtering of all positive reactors.

(2) By the universal pasteurization of all market milk. Since there is evidence to show that pasteurization does alter the milk, it would seem that the interest of health should demand a good, fresh, clean milk at the start.

W.S.M.

710. What an Inspector Should Look for in Making Dairy Cattle Physical Examination. C. U. DUCKWORTH, State Dept. Agr., Sacramento, Calif. Jour. Milk Technol., 4, No. 1: 48. 1941.

An inspector should form the habit upon entering the stable to observe each animal carefully. The physical examination should take in manifest evidence of disease, such as enlarged glands, mastitis, pyometra, tuberculosis, ulcerated teeth, abscesses, and any abnormality in general.

Veterinary council is to be advised where any question occurs with respect to specific nature of any condition found. L.H.B.

711. Experimentelle Brucellose beim Hunde. (Experimental Brucellosis in the Dog.) WALTER DOMKE (Aus dem Hygienischen institut der Tierärztlichen Hochschule Hannover.) Ztschr. f. Infektionskrank., Parasitäre Krank. u. Hyg. der Haustiere, 56, No. 4: 321-328. 1940.

The views concerning the abortus bacillus infection in the dog and the possibility of this animal being a disseminator of the organism are conflicting. The serological evidence on this point is very limited. Some writers on the subject consider that there may be certain factors which will influence the sensitivity of these animals to this infection.

Dogs of different ages were fed and inoculated with the organism. Clinical observations and agglutination tests were made for three-month periods when the animals were killed and cultural and pathological studies made. In order to test the localizations of the organisms suspensions of the sex organs were made in salt solution and 2 cc. injected into guinea pigs. These animals were then examined for 6 weeks when they were killed and cultural and serological examinations made.

From this investigation it was concluded that the dog is susceptible to *Brucella bovis* infections by feeding and injection. Clinically the course of the disease in the dog is much the same as in man. Pathological changes were observed in the genital organs. The agglutination titer followed very closely the fever curve. Gravid females were more susceptible than others.

Pathologically the disease in dogs is similar to that in cattle in that the organisms localized in the placental membranes.

An agglutination titer of 1:80 was observed in some of the infected animals. L.D.B.

712. Erfahrungsbericht über die amtlichen tierärztlichen Milchuntersuchungen aus dem Jahre 1937-1938 in Regierungsbezirk Oberschlesien. (Report of the Official Veterinary Milk Investigations for 1937-1938 in Upper Silesia.) OTTO SCHIEL (Aus dem Staatlichen Veterinär-Untersuchungsamt Oppeln). Ztschr. f. In-

fektionskrank., Parasitäre Krank. u. Hyg. der Haustiere, 57, No. 2: 159-176. 1941.

This issue contains only the first part of the paper; the final part will appear in the next issue of this journal.

The report is detailed and contains considerable information on the results of official investigations of the milk supply of the area together with a scheme for control of milk-borne diseases. The report deals with the finding of tubercle bacilli, the Bang's disease organism and mastitis streptococci.

It has not been possible to control tuberculosis entirely in these larger areas and the official veterinary control service has developed plans for a more detailed study of the disease producing organisms and methods of control in a limited area. The study covered the years 1937 and 1938. Udder tuberculosis was found in 1.92 per cent of 3,381 samples from 61 different sources. Abortion bacilli were found in 8.21 per cent of these samples. Microscopic examination revealed 1.16 per cent contained mastitis streptococci. There was considerable variation in the results obtained with milk from different sources.

Conclusions will appear in the next issue.

L.D.B.

713. Zur Feststellung von Tuberkelbazillen in Ausscheidungsproben vom Rind. (A Study to Determine the Presence of Tubercle Bacilli in the Excreta of Cattle.) W. STOCKMAYER (Aus dem Württ. Tierärztlichen Landesuntersuchungsamt.) Ztschr. f. Infektionskrank., Parasitäre Krank. u. Hyg. der Haustiere, 57, No. 1: 75-84. 1940.

The older methods of detecting the presence of tubercle bacilli in excreta by animal inoculation is tedious, time-consuming and costly. The writer has developed other methods which he considers as satisfactory substitutes. The methods here described are microscopic, cultural and animal inoculation.

The microscopic methods may be direct, using fresh material or following an enrichment. A combination of the two appears preferable to either alone. The writer examined 202 samples of bronchial slime from tuberculous animals by means of the antiformin method using 2.5 per cent concentration of the chemical. The material was obtained with a tracheal cannula and swab. The sample thus obtained by means of a swab was spread directly on slides. The swab was then placed in 2.5 per cent antiformin for 20 minutes and squeezed out with sterile forceps. The fluid thus obtained was centrifuged and the sediment stained by the Ziehl-Neelsen method and examined. Of the 202 samples studied, 119 were positive by direct examination and 113 by the so-called enrichment process. In 41 instances the direct examination was most strongly positive and in 24 instances the

enrichment method gave better results. The reason the direct examinations gave more positive results was that the organisms were in clumps and more easily found. Also, the antiformin treatment tends to reduce the intensity of the staining.

The usual method of injecting animals and holding them for eight weeks with frequent examinations and removal of regional lymphatics is time-consuming. An attempt was made to reduce this period. The material obtained on a swab was placed in 1 cc. salt solution and centrifuged and the sediment suspended in 1 cc. of the cream obtained from 60 cc. of milk. This material was injected into guinea pigs and the animals examined at weekly intervals. When enlarged lymph nodes could be observed they were recovered under local anaesthesia and examined microscopically for the presence of tubercle bacilli. All negative glands were examined histologically and the presence of giant cells was considered as positive. The writer concludes that holding the animals for six weeks is not sufficient.

For cultures the writer used sediments obtained by suspending the slime in an HCl solution (15 cc. HCl sp. gr. 1.122 plus 83 cc. aq. dist.) from five to 15 minutes with shaking followed by centrifugation for 10 minutes. The sediment was then placed on Patragani-Witte media. It was found that this concentration of HCl did not destroy the tubercle bacilli in the time used. There was good agreement between the results of culture and animal inoculation. The culture usually gave 20-30 per cent more positive results than the microscopic examination.

As a result of this investigation the writer concludes that animal inoculation gives the highest results and that the guinea pigs used in such investigations should be held for eight weeks or longer. L.D.B.

FOOD VALUE OF DAIRY PRODUCTS

714. Effect of Milk on Gizzard Erosion and Cholic Acid in the Chick.

H. J. ALMQUIST, E. MECCHI AND F. H. KRATZER, College of Agr., Univ. California, Berkeley. Soc. Expt. Biol. and Med. Proc., 47: 525. 1941.

Evidence was presented for the existence in cows milk of a labile substance which acts like cholic acid. Dried milk products were fed by mixing in the diets while liquid milk products were given to the chicks in place of the drinking water. Liquid milk products reduced the severity of gizzard erosion, increased the gall bladder bile volume per chick and increased the quantity of cholic acid per chick. There was no effect on the characteristics measured following the feeding of dried milk products. Attempts to detect cholic acid in skim milk produced only negative results.

R.P.R.

715. **The Effect of Vitamin A Intake on Vitamin A Content of Butterfat.** HARRY J. DEUEL, JR., NELLIE HALLIDAY, LOIS HALLMAN, CORNELIA JOHNSTON AND ALBERT J. MILLER, Dept. of Biochemistry, Univ. of Southern California, Los Angeles, Calif. *Jour. Biol. Chem.*, 139, No. 1: 479. May, 1941.

The supplementary feeding of vitamin A in the form of Shark liver oil greatly increased the vitamin A content in the butterfat. The milk production of the cows receiving the vitamin A supplement promptly rose and continued at a level approximately 10 per cent higher than the cows not receiving the vitamin A during the test. V.C.S.

716. **Proteins and Our Dairy Products.** F. H. PLETCHER, Lab., Borden Farm Products, Brooklyn, N. Y. *Milk Dealer*, 30, No. 8: 36, 56-60. May, 1941.

Proteins are discussed under the following headings: Why Stress Proteins? What is Protoplasm? Chemical Composition. Amino Acids. Milk Proteins. Needs of Adults. The author summarizes his discussion as follows:

Why stress the importance of proteins in our dairy products when there are also present the all important minerals, and excellent carbohydrate, butterfat and vitamins in varying quantities? Possibly because we seldom realize we handle this food nutriment—when we think of milk our co-thought is usually fat and sometimes total solids.

Proteins, however, are highly important in the diet—especially the milk proteins, for in milk we have the “complete” proteins, capable of maintaining life and supporting growth. Other foods are rich in protein, but they are usually lacking in some essential amino acid and are therefore either “incomplete” or “partially incomplete.” An example of a food rich in “incomplete” proteins is gelatine, which will neither maintain life nor support growth; cereals, on the other hand, are generally classed as “partially incomplete” since they are usually lacking in at least one essential amino acid which is necessary for the promotion of growth, although they will maintain life.

But in milk or other dairy products such as cottage cheese we have protein foods containing all of the known 23 amino acids which when included in the dietary, in sufficient amounts, have been proved by experimentation to possess outstanding properties.

It's up to the men in the dairy industry to educate consumers on this point. C.J.B.

717. **Effect of Supplemented Raw and Pasteurized Milks upon Growth and Well-being of Rats.** ALICE M. BAHRS, St. Helen's Hall Junior

College, Portland, Ore., AND ROSALIND WULZEN, Orgeon State College, Corvallis, Ore. *Certified Milk*, 16, No. 180: 5. Apr., 1941.

Rats fed raw milk rations showed a superior growth and certain tissues of the body showed distinct differences. W.S.M.

718. **The Soil Basis of Better Milk Production.** L. A. MAYNARD, Cornell Univ., Ithaca, N. Y. *Certified Milk*, 16, No. 181: 5. May, 1941.

The author discusses ways in which the soil is definitely related to milk quality. It is generally understood that the nature of the feed of the cow affects the quality of the milk as well as its quantity. But, it is less appreciated that the quality factor in the feed depends upon how the feed crop is produced and particularly upon the fertility of the soil. The influence of the soil on milk quality is an indirect one, expressing itself through the food crop, yet it has some advantages over adding deficient nutritive essentials directly to the feed. W.S.M.

719. **Possibilities of Improving Milk by Increased Nutritive Qualities in Feeds.** D. B. HAND, Cornell Univ., Ithaca, N. Y. *Certified Milk*, 16, No. 182: 7. June, 1941.

The author concludes that iodine and a few of the fat soluble constituents, notably vitamins A and D, are the only substances in milk which are greatly dependent on feed. From the practical standpoint for some of the other constituents, it is possible to improve the quality of milk by selection of individual cows. However, selection of cows offers many complicated problems. The quality of milk with respect to color and flavor can be improved by feeding. How many compounds are present in milk and which of these are essential to human diet is still unknown. W.S.M.

720. **Nutritional Restoration and Fortification of Foods.** Jour. Indus. and Engin. Chem., 33, No. 6: 707-722. 1941.

Several papers on this subject which are of general interest were presented in a symposium at the 101st meeting of the American Chemical Society, St. Louis, Missouri. These papers are as follows:

Nutritional Requirements of Man. C. A. Elvehjem, Univ. of Wisconsin, Madison, Wis.

Cereal Products. R. T. Connor, General Foods Corp., Hoboken, N. J.

Fortification and Restoration in the Baking and Dairy Industries. James A. Tobey and William H. Cathcart, Amer. Inst. of Baking, New York and Chicago.

What the Consumer Should Know about Fortified Foods. Helen S. Mitchell, Nutrition Div., Health, Welfare and Activities Affecting National Defense, Washington, D. C.

Fortification and Restoration of Processed Foods. R. R. Williams, Bell Telephone Labs, New York, N. Y.

Control Problems of the National Nutrition Program. E. M. Nelson, U. S. Food and Drug Admin., Washington, D. C. B.H.W.

ICE CREAM

721. **Helping the Ice Cream Retailer Stay in Business.** JOHN KIRKWOOD, Advertising and Marketing Counsellor, Toronto, Ont. Canad. Dairy and Ice Cream Jour., 20, No. 2: 52. 1941.

The author discusses rather lengthily the principles of a retail business. Although he points out that 75 per cent of retailers do not last 7 years, he states that retailing can be a "safe" business if business principles are charted and the chart used as a guide. O.F.G.

722. **Some Suggestions for Stepping up Winter Sales of Ice Cream.** ANONYMOUS. Ice Cream Rev., 24, No. 7: 94. 1941.

Suggestions are given for increasing the use of ice cream during the winter months by means of health appeal, colored mailing pieces and magazine advertisements. J.H.E.

723. **Ice Cream Production by Regions for the Years 1930-1939.** E. E. VIAL, Bureau of Agr. Econ., U. S. D. A., Washington, D. C. Ice Cream Rev., 24, No. 7: 84. 1941.

Data, gathered by the Agricultural Marketing Service, is compiled showing ice cream production and per capita consumption for principal geographic areas. These data indicate that per capita consumption of commercial ice cream is highest in the North Atlantic states, amounting to 3.02 gallons in 1939. The South Central states were lowest with 1.17 gallons per capita in the same year. J.H.E.

724. **Sugars That Can Be Used in Ice Cream Making.** P. H. TRACY, Univ. Illinois, Urbana, Ill. Canad. Dairy and Ice Cream Jour., 20, No. 2: 68. 1941.

Sugars, which compose approximately 20 per cent of the weight of ice cream, perform several rather important functions when used in ice cream. They are high in energy value and greatly increase the palatability of the ice cream. They sufficiently lower the freezing point of the mix to permit incorporation of the desired amount of air without the semifrozen mass becoming too stiff to be removed from the freezer. Granulated cane and beet sugars are the most important sources of sweetness for ice cream. Generally the dry sugar has been used but in recent years a sugar syrup which contains about 68 per cent solids has found some favor. The advantages of the syrup are that it can be transported in tank trucks and

handled by pumps. Corn sugar, known as dextrose, glucose or cerelese, is a monosaccharide and differs in some of its properties as compared to cane or beet sugar, known as sucrose, which is a disaccharide. Dextrose is manufactured by hydrolyzing corn starch. It has a sweetening value of approximately 70 as compared to 100 for sucrose but its sweetening power is increased when used in conjunction with sucrose in ice cream. A new type of corn syrup, known as "Sweetose," recently has been perfected which has a much higher dextrose equivalent and better flavor than the older syrup. "Sweetose" has a beneficial effect upon the body of ice cream, sherbets and ices. A dry corn sweetening agent, known as "Fro-dex," has recently been introduced. In the selection of a sweetening agent the manufacturer of ice cream should be governed by cost as well as by the effect of the product upon the ice cream. O.F.G.

725. Some Pointers in Making Sherbets and Water Ices. P. H. TRACY, Univ. Illinois, Urbana, Ill. *Canad. Dairy and Ice Cream Jour.*, 20, No. 3: 20. 1941.

Desirable features of a good stabilizer for ices and sherbets are given as follows: (1) Stabilizing qualities should not be impaired by citric acid, (2) should be easily dispersed, (3) should be a desirable effect upon texture and resistance, (4) should have sufficient effect upon viscosity to prevent settling out of unfrozen syrup, (5) should not cause high overrun, (6) should be tasteless.

The merits and methods of using such stabilizers as gelatin, gums, pectin and carob bean products are discussed. The amount of a monosaccharide sugar, such as honey, dextrose or sweetose, which can be used in conjunction with sucrose is limited to about 7 per cent. The following defects are discussed: bleeding, surface crustation, crumbly body, hard body, snowy body, coarse body and sticky body. O.F.G.

726. Ice Cream Sales Index for 1941. Statistical and Accounting Bureau. Internatl. Assoc. Ice Cream Mfrs., Washington, D. C. July, 1941.

This bulletin contains an analysis of ice cream sales in the United States and Canada for the first four months of 1941. The increase in sales over 1940 for the first four months of 1941 is as follows:

Month	Per cent of increase	
	United States	Canada
January	31.42	49.07
February	9.57	39.07
March	15.14	45.08
April	27.66	78.44
Average increase	21.05	56.52

The Central Eastern States led the increase for the four-month period with a gain of 28.4 per cent, the North Atlantic States, followed with 21.67 per cent increase over the previous year, the Midwestern States enjoyed a gain of 17.59 per cent, the Southern States 15.77 per cent, while in the Western States the increase was 4.76 per cent.

The bulletin also contains an analysis of business and weather conditions in the different sections of the country. In the supplement to the bulletin the final ice cream statistics for 1939 of the Agricultural Marketing Service of the U.S.D.A. are given. The total production of ice cream for 1939 was 304,522,000 gallons.

M.J.M.

727. **The Frozen Desserts Code Recommended by the Public Health Service.** A. W. FUCHS, Sanitation Section, U. S. P. H. Service, Washington, D. C. *Jour. Milk Technol.*, 4, No. 1: 26. 1941.

The need for public health control of frozen desserts is cited. Prior to 1928 there have been 36 outbreaks of milk borne disease reported in the literature as having been traced to ice cream. In the five-year period, 1934 to 1938 inclusive, ten outbreaks have been reported.

The Public Health Service urges states not already doing so to adopt a frozen desserts control program similar to the milk control program.

L.H.B.

728. **Chocolate Malted Milk.** C. E. HENDERSON, Bastian-Blessing Co. *Ice Cream Rev.*, 24, No. 6: 31. 1941.

The popularity of chocolate malted milk drinks is due to their delicious taste, high food value and the quick energy they provide. The variable factors are the chocolate flavor and the consistency of the finished drink. The consistency depends not only upon the proportions of milk and ice cream used, but also upon the temperature of the ingredients and the mixing time. Blending is done by whipping air into the milk as the ingredients are mixed. Milk at 32° F. can hold approximately 90 per cent air. Electric mixers accomplish mixing and aeration in less than two minutes. If the mixing is too rapid, the ice cream will be broken down instead of blended. If not removed from the mixer when the maximum aeration of the milk has been accomplished, the air will escape as the temperature of the milk rises. Success consists largely in keeping the ingredients as near the freezing temperature as possible during mixing.

A number of formulas are given.

J.H.E.

729. **New Developments in the Science of Ice Cream Making.** W. H. MARTIN, Kansas State College, Manhattan, Kan. *Ice Cream Rev.*, 24, No. 6: 34. 1941.

This is a review of the recent problems and developments in ice cream

manufacture. Shrinkage, ingredients, melting qualities, stabilizers, flavors and sanitary quality are some of the subjects discussed. J.H.E.

730. **Sugars That May be Used in the Ice Cream Industry.** P. H. TRACY, Univ. of Illinois, Urbana, Ill. *Ice Cream Rev.*, 24, No. 5: 29. 1940.

When the ice cream manufacturer replaces sucrose with other sweetening agents he should expect some differences in results. The type of sweetness may not be the same, but this is not necessarily a disadvantage. Some of the present replacement agents contain prosugars of high molecular weight so that the lowering of the freezing point of the mix is not a serious problem. It is claimed that in some cases a better flavor and a better body may result from the use of a sweetening agent made from corn. J.H.E.

731. **The Control of Shrinkage in Ice Cream.** J. H. ERB, Ohio State University, Columbus, Ohio. *Canad. Dairy and Ice Cream Jour.*, 20, No. 3: 60. 1941.

The mechanism of ice cream shrinkage is fundamentally a problem of a destabilized protein or a protein sensitive to coagulation. Factors which operate to influence protein stability are, (1) salt balance of the original milk, (2) added salts, (3) acidity, (4) composition of the mix, (5) homogenizing pressures, (6) stiffness of freezing, and (7) hardening temperature and fluctuations in storage temperature. Another factor which influences shrinkage is the ease of air transfer. Mechanical factors which influence air transfer are (1) type of container, (2) jolting of truck, (3) amount of overrun, (4) size of air cells, and (5) external pressures. Factors making for a stable, or highly hydrated plastic protein are desirable in correcting shrinkage since such a protein better holds the air within the cells. O.F.G.

732. **Is There a Place for Substandard Products in the Ice Cream Industry?** C. H. SNOW, Snow and Palmer Co., Bloomington, Ill. *Ice Cream Rev.*, 24, No. 5: 74. 1940.

There are dairy products which are meritorious for which standards are desirable. Among these are "cereal cream" and a frozen malted milk mixture. Standards are created by custom of consumers and of the trade generally or they may be created by law.

The test of value in any specific product would seem to be whether or not its production and sale resulted in benefit to the producers of milk and the consumers of the product. Substandard or inferior commodities should not be substituted for the genuine, but there are places for additional standards for special products that fill a real need which is beneficial to industry.

J.H.E.

733. Use of Vanilla and Other Flavors in Ice Cream. ANONYMOUS. Ice Cream Rev., 24, No. 7: 114. 1941.

This is a review of a paper written by Dr. A. Katz of Florasynth Laboratories, Los Angeles, California. In the inception of the ice cream industry, the ice cream was flavored with chopped vanilla beans, as the art of making extract was not known. In the early days of making vanilla extract 95 per cent alcohol was used. This destroyed the fine vegetable aromatic principles present in the vanilla beans. Best results are now obtained using 30 to 35 per cent alcohol.

To obtain proper results in citrus flavorings it is necessary to utilize not only the juice of the fruit but also flavor obtained from the peel. These should be combined together in a vegetable gum media.

Interesting facts about other flavors for ice cream such as butterscotch, English toffee, grenadine, etc., are included. J.H.E.

734. Dextrose and Corn Syrup for Frozen Desserts. A. C. DAHLBERG AND E. S. PENCZEK, New York Agr. Expt. Sta., Geneva, N. Y. Ice Cream Rev., 24, No. 7: 38. 1941. (This is a review of the original New York Agr. Expt. Sta. Bul. No. 696, "Dextrose and Corn Syrup for Frozen Desserts" by the authors of this article.)

Good results can be secured when 25 per cent of the sucrose in ice cream is replaced with dextrose or corn syrup to give comparable sweetness. Based upon securing comparable sweetness the weight of the dry corn sweeteners required to replace one pound of sucrose is as follows: Enzyme-converted corn syrup, 1.5 pounds; corn syrup solids, 2.0 pounds, and dextrose, 1.1 pounds. J.H.E.

735. Understanding Improves Consumer Friendship. RACHAEL REED, The Borden Co., Chicago, Ill. Ice Cream Rev., 24, No. 7: 34. 1941.

It is possible for the ice cream industry to profit by the experience of fluid milk and other industries in giving the consumers the information they would like to have about the ice cream industry and its products before they become too critical. A number of things consumers are interested in about ice cream are discussed. The article contains several good tables on the comparative food value of ice cream and other desserts. J.H.E.

736. High Operating Costs. C. F. BAKER, Atlanta, Ga. Ice Cream Rev., 24, No. 7: 29. 1941.

Some of the common causes for high operation costs of the refrigeration system are malpractices in connection with pumping condenser water, low ammonia charge, high condensing pressures and oil in evaporating coils. Examples of these conditions and their remedy are given. J.H.E.

737. Cherries, the Luxury Fruit. HOWARD BLACK, Traverse City, Mich. Ice Cream Rev., 24, No. 7: 26. 1941.

The author sketches some interesting history of cherries. Cherries are a valuable ice cream flavor because of the eye appeal. J.H.E.

738. Formulas for Combination Flavors of Pineapple and Other Flavors. ANONYMOUS. Ice Cream Rev. 24, No. 6, 52. 1941.

A number of new ice cream flavors combining pineapple and other popular flavors has been developed by Dr. C. D. Dahle, of Pennsylvania State College. Detailed formulas and other helpful instructions are given for a number of combinations. J.H.E.

739. Pasteurizing the Ice Cream Mix. J. M. BRANNON, Univ. of Illinois, Urbana, Ill. Ice Cream Rev., 24, No. 6: 28. 1941.

The ice cream industry is endeavoring to fix a standard temperature and time for pasteurization of ice cream mix. It has been shown that a temperature of 150° F. for three minutes will kill *Eberthella typhi*, beta hemolytic streptococci, *Corynebacterium diphtheria* and bovine tubercular bacilli in ice cream mix. Twelve states have set bacterial standards for ice cream.

The results of a survey of Illinois ice cream made a few years ago by the author are cited which indicated only 17 per cent of the samples had bacterial counts of 100,000 or less. Sixty per cent of these samples gave a positive test for the coli aerogenes group. The author concludes that in the average plant the ice cream mix is generally sufficiently pasteurized but that large numbers of organisms are picked up from equipment after pasteurization. J.H.E.

740. Cutting Truck Refrigeration Costs. ANONYMOUS. Ice Cream Rev., 24, No. 6: 26. 1941.

The experience of a southern ice cream company with a new group of five refrigerated trucks is discussed. Three of the five trucks are used for city delivery and are quipped with ammonia refrigeration coils. These are hooked up each day to take-off lines at the plant. Trucks on country routes, which do not return to the main plant every day, are equipped with compressors as well as power take-off units on the drive shaft. Savings have been especially realized for the country trucks because they do not lose efficiency when kept in the territory overlong. They can spend more time in actual service than formerly. J.H.E.

MILK

741. Milk in the Schools. F. W. HAMILTON, Royal Oak Dairy, Ltd., Hamilton, Ont. Canad. Dairy and Ice Cream Jour., 20, No. 1: 17. 1941.

The primary objective in the sale of milk in schools is not the sale of milk

but the development of the milk drinking habit. The school is the best place to develop in young people the habit of drinking milk instead of soft drinks, tea and coffee. A plan was worked out through the Board of Education whereby the dairies agreed to furnish refrigeration in the schools and in return were to be allowed to sell "free" milk to school children. The schools were apportioned to those competing dairies which wished to enter the plan. For the year 1938-1939, before the plan was put into effect, the amount of free milk given out was 273,000 half pints. For the year 1939-1940, after the plan went into effect, the free milk totaled 378,000 half pints and the sale milk totaled 285,000 half pints. O.F.G.

742. **Cooked Flavor in Milk, a Study of its Cause and Prevention.** I. A. GOULD, Michigan State College, East Lansing. Internatl. Assoc. Milk Dealers, Assoc. Bul., 33rd yr., 21: 553-564. Apr., 1941.

That the "sulfurous-like" flavor produced in milk by high temperature heat treatment is related to sulfur compounds is shown by producing the defect by adding to milk a sulfite salt or glutathione which contains the sulfhydryl (-SH) linkage and artificially producing a cooked flavor. Two methods were employed to determine if sulfur were involved (a) liberation of sulfides from the milk, (b) the nitro prusside test which detects the presence of sulfhydryl (-SH) groups for which a slight modification of the technique of Jacobson and Doan was used. The temperature at which the flavor appears is 76-78° C. (169-172° F.) for momentary heating and 70-72° C. (158-162° F.) for 30 minute holding.

The momentary temperature required to produce the cooked flavor is raised to 84-86° C. (183-187° F.) when 1 p.p.m. of copper was added after heating. A somewhat closer relationship was found between the cooked flavor and the sulfhydryl groups than heat labile sulfides. A lower critical temperature for cream than skim milk suggests that the proteins are associated with the fat, also these proteins were not removed by 3 washings of the cream. The retardation or prevention of oxidized flavor in cooked milk may be due to the creation of a reducing system unfavorable to oxidation or through direct combination with the metals which are oxidative catalysts. Copper is more effective in preventing or dispelling cooked flavor when added after heating. Two p.p.m. of copper has been used to dispel heated flavor in heat treated soft curd milk. E.F.G.

743. **Developments in Production and Reports in 1940.** J. F. SINGLETON, Assoc. Director of Marketing Service, Dairy Products, Ottawa, Ont. Canad. Dairy and Ice Cream Jour., 20, No. 1: 24. 1941.

The production and marketing of dairy products in Canada for 1940, has been conditioned greatly by the needs of the United Kingdom. There has been some diversion of milk supplies from butter to cheese and evaporated

and condensed milks. Butter prices dropped while cheese prices increased. In order to conserve supplies of cheese for the Ministry of Food, the Board has taken action to restrict exports to non-Empire countries. Production of condensed milk, evaporated milk and skim milk powder was higher than in 1939. Greater total milk production for 1941 could be used to advantage.

O.F.G.

744. **Operating a Credit Bureau in the Milk Industry.** E. J. LeBOEUF, Windsor Milk Distributor's Assoc., Windsor, Ont. *Canad. Dairy and Ice Cream Jour.*, 20, No. 1: 44. 1941.

The Credit Collection Agency results in a control bureau which does away with loose credits by keeping accounts from becoming inactive. The co-operative method promotes good will to all.

O.F.G.

745. **Co-operation for Efficient Milk Production.** H. B. ELLENBERGER, Univ. Vermont, Burlington, Vt. *Canad. Dairy and Ice Cream Jour.*, 20, No. 2: 20. 1941.

Milk can be made more cheaply when approved modern and efficient methods are practiced more generally on farms. Both co-operatives and proprietary distributors can well afford to lend a helping hand to producers, for in the long run both would profit. Many reasons exist why distributors and producers should co-operate. It is net income rather than gross income that is usually important and efficiency is the master key to better net income. Dealers and producers can accomplish more through working jointly than through independent action.

O.F.G.

746. **The Determination of the Viscosity of Human Milks and the Prenatal Secretions.** GEORGE W. SCOTT BLAIR, Natl. Inst. Res. in Dairying, Univ. Reading, Reading, Eng. *Biochem. Jour.*, 35, No. 3: 267-271. March, 1941.

An apparatus, quite similar in principle to the Ostwald pipette, is described by which the viscosity and viscous anomalies of small (1 ml.) samples of human mammary secretions may be quickly and accurately determined.

These secretions, tested at blood heat, behave in general, surprisingly like true fluids, i.e., their viscosities differ but little with varying shearing stress.

V.C.S.

747. **Methods of Producing High Quality Table Cream.** O. J. SCHRENK, Bowman Dairy Co., Chicago, Ill. *Canad. Dairy and Ice Cream Jour.*, 20, No. 2: 58. 1941.

All the rules and regulations laid down for the production of good milk should apply to cream. Cleaned, rinsed and sterilized equipment made of

metals that cause no off-flavors is essential to good flavor in cream. Good viscosity is essential to table cream and methods of producing a higher viscosity are described. The chief contributing factor to undesirable cream plug formation is agitation at temperatures within the churning range. Cream feathering is increased by, (1) coffee made with hard water, (2) long-time contact of coffee with the grounds, (3) high acid cream, (4) high calcium and magnesium content of cream, (5) low pasteurization temperature, (6) high homogenization pressure, (7) low homogenization temperature, and (8) single-stage homogenization. Feathering is reduced by (1) soft water, (2) short-time contact with coffee grounds, (3) low acid in cream, (4) addition of sodium citrate, (5) high pasteurization temperature, (6) low homogenization pressure, (7) high homogenization temperature, and (8) two-stage homogenization. The formation of a skim milk layer may be inhibited by high pasteurization temperature or careful homogenization. To the average consumer yellow color in the cream is an indication of richness and therefore is important. O.F.G.

748. **A Discussion of Public Relations in the Milk Industry.** H. L. GARNER, Ontario Daily Newspaper Assoc., Peterborough, Ont. *Canad. Dairy and Ice Cream Jour.*, 20, No. 2: 26. 1941.

A public relations program really means a program for building public goodwill, or favorable public opinion. To sell your milk products, you must, first, win the goodwill of the public and, second, win the good opinion of the workers in your plant. Goodwill is based on good "works" and on good "words." O.F.G.

749. **Public Relations in the U. S. Milk Industry.** G. G. DIFFENBACH, Abbott's Dairies, Philadelphia, Pa. *Canad. Dairy and Ice Cream Jour.*, 20, No. 3: 23. 1941.

A vast amount of work by the industry along nutritional and consumption lines is essential. An intensive type of activity must be pursued to acquaint the public with the unique position of the fluid milk distributor and the industry he represents. People must be informed more fully about the functions and operation of the dairy industry. O.F.G.

750. **Mechanical Principles and Problems of Vat Pasteurization.** A. H. RISHOR, Cherry Burrell Corp., Chicago, Ill. *Dairy World*, 20, No. 1: 22. June, 1941.

This is a detailed discussion of the problem of heat transfer in vats used for pasteurizing milk products particularly with reference to conductivity of the material separating the heating or cooling medium and the fluid being heated or cooled, the temperature gradient and the importance of agitation

on either side of the material through which the heat flows. The following conductivity table is presented:

Material	Temperature °F.	Conductivity
Aluminum	64	0.504
Brass	64	0.260
Copper	64	0.918
German silver	32	0.700
Iron	64	0.161
Steel	64	0.115
Stainless steel	212	0.039
Nickel	64	0.142
Tin	64	0.155
Glass	68	0.002
Water	68	0.00143
Air	32	0.0000568

F.J.D

751. **Pasteurization of Modified Milk Products.** K. G. WECKEL, Univ. Wisconsin, Madison, Wis. Dairy World, 20, No. 2: 17. July, 1941.

Pasteurization treatments for milk "by-products" differ from that for milk since body or viscosity, texture, flavor, solution of ingredients, color, bacteriological effects, etc., are often extremely important considerations, whereas with milk the main concern is to render the product free of pathogens. The author discusses the pasteurization or heat treatment problem involved in the manufacture of buttermilk, cultured cream, chocolate milk, pasteurized cream, homogenized milk, cheese spreads, ice cream mix and some less important products.

F.J.D.

752. **Oxygen Constant Variants.** E. S. GUTHRIE, PAUL F. SHARP AND DAVID B. HAND, Cornell Univ., Ithaca, N. Y. Amer. Milk Rev., 2, No. 6: 131, 132. June, 1940.

Milk absorbs from 3.84 to 9.74 p.p.m. of oxygen during hand milking but contains none while in the udder. It is absorbed slowly when the surface is quiet, is driven out during vat pasteurization, and reabsorbed during cooling and bottling. If deaerated it may be bottled without additional air by admission to the bottom of the bottle through a tube, taking care to cause a minimum of agitation.

P.S.L.

753. **Testing Pasteurized Cream.** HERBERT JENKINS. Amer. Milk Rev., 3, No. 3: 58-60. March, 1941.

Seeking to cut the time required for plate counts of pasteurized cream, many tests using resazurin dye were run, the time required being 6 to 7 hours. Those creams having a reduction time of 6 to 7 hours, when plated, had a plate count of less than 40,000 bacteria, the average being 17,000. Such cream occasioned no complaints from customers.

P.S.L.

754. **Studies on Soft Curd Milk Prepared by the Enzyme Treatment Method.** A. W. TURNER, University of Illinois, Urbana, Ill. Soc. Expt. Biol. and Med. Proc., 46: 593. 1941.

An investigation was made of the characteristics of enzyme-treated milk. The enzyme treatment was carried out by adding one part of pancreatic concentrate to 10 to 15 thousand parts of cold raw whole cow milk followed by immediate pasteurization. Enzyme-treated milk contained more 70 per cent alcohol-soluble protein and proteose peptone nitrogen but only slightly more amino nitrogen than ordinary pasteurized milk. Casein prepared from enzyme-treated skim milk was more soluble in 70 per cent alcohol than was casein prepared from pasteurized skim milk. R.P.R.

755. **Are You Encouraging the Love Life of a Fly?** ANONYMOUS. Milk Dealer, 30, No. 8: 76-80. May 1941.

A discussion is given on how to control the fly in dairy plants. The article is summarized as follows: The best arrangement for controlling the fly menace, it would seem, is first to clean up and keep clean the premises; second, screen all doors and windows; third, supplement screens with electric screens to kill flies seeking admission; fourth, use a good power spraying system to get rid of the flies which get into the plant. C.J.B.

756. **The Contribution of Industrial Milk Service to National Defense.** JAMES R. HUDSON, Baker-Stuber Dairy, Peoria, Ill. Milk Dealer, 30, No. 8: 116-121. May 1941.

A discussion is given of how the dairy industry can contribute to national defense by supplying factory workers with enough milk to keep up their efficiency, reduce absenteeism, and maintain their morale. C.J.B.

757. **The Instantaneous Heat Treatment of Milk.** G. C. SUPPLEE AND O. G. JENSEN, Borden Co., Bainbridge, N. Y. Jour. Milk Technol., 4, No. 1: 5. 1941. (Also published in the Annual Proceedings of the New York State Assoc. of Dairy and Milk Insp., 1940.)

Using a flowing film electric pasteurizer the authors studied the effect of momentary exposure periods at various temperatures on bactericidal effectiveness, flavor, cream line and phosphatase test.

Exposure periods as short as 0.8 second between the lethal temperature range of 145° F.-185° F. were found effective in bactericidal reduction. No automatic controls were used on the equipment either to regulate the temperature or the flow of current. Variations in temperature from the average operating level did not exceed about 3°, and it was believed that the major variations may have been due to surges in the feed line voltage.

It was found that 76 per cent of the milks exposed to 180° F. and above showed a percentage reduction in bacterial count of over 99 per cent.

Data was obtained on the phosphatase test using Gilcreas and Davis' modification for average or mean temperatures of 163°, 173°, 177°, 181°, and 186° F.

At 163° F. all samples were classified as grossly underpasteurized, showing values of 0.15 and above. At 173° F. they varied from 0.03 to 0.15. At 177° F. they varied from 0.00 to 0.09; about 70 per cent of the samples were classified as satisfactorily pasteurized and the remainder as slightly underpasteurized. At 181° F. and above they were all classified as satisfactorily pasteurized having values of 0.04 or less.

The characteristic heated milk flavor was practically undetectable at operating temperature of 185–186° F. Improvement in the flavor was even noted at operating temperature of 185° F. and lower. At 190° F. the heated flavor was detected by experts, but was not as pronounced as is frequently observed in milk pasteurized by usual methods.

Reduction in creaming ability when compared to the raw milks ranged from 6 to 20 per cent through the temperature range of 160° F. to about 180° F. Creaming ability was destroyed most rapidly per degree increase in temperature through the 185–190° F. range.

L.H.B.

758. A Simplified Procedure for Laboratory Examination of Raw Milk Supplies. R. P. MEYER AND J. A. PENCE, Sealtest, Inc., Baltimore, Md. Jour. Milk Technol., 4, No. 1: 18. 1941.

For the purpose of testing producers' milk to find if it contains thermocidic organisms, the use of the loop measurement, oval tube technique saves time and material in making the laboratory pasteurization tests.

Test briefly is as follows:

Pasteurize 5-cc. samples of milk in screw-capped vial at 143° F. for 30 minutes, in a constant temperature bath, cool, shake vigorously 50 times. With standard loop needle (0.001 or 0.01 cc.) transfer loopful of milk to an oval culture tube (size 152 mm. in length and 23 mm. by 11.5 mm. in diameter) containing approximately 4 cc. of sterile melted T.G.E. agar cooled to 45° C. Mix contents by swinging tube through a small arc for about 5 seconds. Tube is then laid on table with open end raised about $\frac{1}{8}$ inch so tube is slanted to permit agar to flow to a point 2.5 to 3 inches from bottom of tube. Allow agar to harden. Place tubes in special wire rack in horizontal position with agar adhering to upper side of tube. Incubate for 48 hours at 37° C. Count colonies in usual manner by placing tubes over a well lighted colony counter. This method is reported to save one-half the time, uses only $\frac{1}{8}$ as much agar, and requires no dilution blanks and pipettes.

Results checked very closely with standard agar plate count using T.G.E.M. agar.

L.H.B.

759. To What Extent Should Bacterial Counts of Milk be Given Publicity. C. C. PROUTY, Agr. Expt. Sta., Washington State College, Pullman, Wash. Jour. Milk Technol., 4, No. 1: 32. 1941.

Bacterial counts should not be given publicity on the ground that the milk consuming public is not aware of the limitations of making bacterial counts, and therefore, not qualified to interpret them properly in relation to the sanitary quality of the product.

Equal ratings should be given to all samples falling into the same count bracket.

A discussion of the paper by M. E. Parker, of the Beatrice Creamery Company, Chicago, Illinois, is also given. L.H.B.

760. "Approved Milk" for New York City in Place of Grade A and Grade B. J. L. RICE AND SOL PINCUS, N. Y. City Dept. Health. Jour. Milk Technol., 4, No. 1: 38. 1941.

A history of New York City's milk regulations are given reviewing the events in the development of milk control which leads up to the latest step of eliminating A and B grades and replacing with "Approved Milk."

The standards for the "approved milk" are stricter than formerly called to grade B milk which was the bulk of their supply. A chart showing the former requirements for grade A and B is given comparing the standards with those of "approved milk."

Advantages expected to be gained by simplification of grading system are:

1. It will be possible to concentrate all energies upon improving general supply without regard to grading.
2. With the elimination of dual grading the consideration of grade A as the only safe milk was removed as was the inferiority implication given grade B. Confidence of public in their milk supply will be encouraged.
3. Sanitary control will be simplified and the industry will be enabled to eliminate some plant duplication, where formerly they were required to maintain separate equipment for handling grades A and B. L.H.B.

761. Report of Committee on Applied Laboratory Methods. T. H. BUTTERWORTH, San Antonio, Texas. Jour. Milk Technol., 4, No. 1: 44. 1941.

The committee presents a program of work for the coming year. At least one member of the committee is actively interested in one or more of the subjects which are as follows:

1. A study of requirement recommendations for officially certified milk and milk products analysis laboratories.
2. A study of the relationship of laboratory tests to field inspection work and an evaluation of the emphasis to be placed on each.

3. A study of the use of the reductase test in controlling raw-to-plant milk supplies.

4. A study of the tentative 32° C. temperature requirements for milk and milk produce incubation.

5. A study of the numerical bacterial content of city supplies of raw-to-plant milk and the best tests for estimating same.

6. By means of a questionnaire to the industry and control officials, a study of the present usefulness of the phosphatase test and the most valuable modification for general use. L.H.B.

762. Some Practical Applications of Milk Technology. E. EUGENE CHADWICK, Acting City Sanitarian, Astoria, Ore. *Jour. Milk Technol.*, 4, No. 1: 45. 1941.

A discussion is given of how two cities in Oregon improved the sanitary conditions of their milk supplies. Milk consumption has increased from 0.7 pint per person to 1.3 pints. L.H.B.

PHYSIOLOGY

763. Growth of the Lobule-Alveolar System of the Mammary Gland with Pregneninolone. JOHN P. MIXNER AND CHARLES W. TURNER, Univ. of Missouri, Columbia. *Soc. Expt. Biol. and Med. Proc.*, 47: 453. 1941.

The injection of pregnenolone either alone or in conjunction with estrone into spayed virgin mice caused the development of the lobule-alveolar system of their mammary glands a property similar to progesterone. Injected estrone enhanced the activity of the pregnenolone about 5 times while under similar conditions progesterone was found to be about twice as effective. R.P.R.

764. Death of Embryos in Guinea Pigs on Diets Low in Vitamin E. ALWIN M. PAPPENHEIMER AND MARIANNE GOETTSCH, Columbia Univ., New York. *Soc. Expt. Biol. and Med. Proc.*, 47: 268. 1941.

A diet low in vitamin E supplemented by 5 to 10 mg. of alpha-tocopherol protected 5 guinea pigs against muscular dystrophy but the amount of supplied vitamin was not adequate to insure successful pregnancy. Three pigs receiving 5 mg. of alpha-tocopherol had resorption at about 30 days and of 2 pigs receiving 10 mg. one gave birth to a living young, followed by resorption (initial fertility) and the other one went beyond mid-term, dying on the 47th day. R.P.R.

765. Effect of Desoxycorticosterone on Pituitary and Lactogen Content. C. W. TURNER AND JOSEPH MEITES, Univ. of Missouri, Columbia. *Endocrinology*, 47: 232. 1941.

Three groups of female and 2 groups of male guinea pigs were injected

with 7 to 20 mg. of desoxycorticosterone acetate over a period of 10 to 20 days to determine its effect on pituitary lactogen content and pituitary weight. The treatment produced a significant increase in pituitary weight but the lactogen content was not altered.

R.P.R.

766. Biennial Reviews of the Progress of Dairy Science. Section A. Physiology of Dairy Cattle. I. Reproduction and Lactation. Jour. Dairy Res., 12, No. 1: 78-107. 1941.

A review of recent literature under the subheadings: Hormones, Biochemical Aspects, Anatomical Aspects, Clinical Chemistry, Climatic and Other Factors Affecting Milk Secretion is given with 208 references.

S.T.C.

767. Some Experiments on the Chemical Enrichment of Cows' Milk by the Administration of Diethylstilbestrol and Its Dipropionate. S. J. FOLLEY, H. M. SCOTT WATSON AND A. C. BOTTOMLEY, Natl. Inst. Res. Dairying, Univ. Reading, Reading, Eng. Jour. Dairy Res., 12, No. 1: 1-17. 1941.

The results secured were extremely variable as is shown by the following summary:

1. Diethylstilbestrol administered orally to a Shorthorn cow had no marked effect on milk yield or composition.

2. A series of injections of the dipropionate in oily solution led to a slight rise in non-fatty solids in the same cow as in (1). Single larger injections were followed by a rise in milk solids accompanied by a rapid fall in milk yield in two other Shorthorns.

3. Inunction with an ointment containing the dipropionate led to a marked increase in milk solids in a Shorthorn cow, with no change in milk yield. The effect subsided rapidly when treatment was stopped. No significant effects were produced by similar treatment of four pregnant British Friesians; on increasing the dose two of these aborted. A Guernsey cow showed a slight increase in non-fatty solids and a slight, but temporary, fall in milk yield.

4. Subcutaneous implantation of crystalline diethylstilbestrol led to a striking and prolonged increase in milk solids, with no fall in milk yield, in a Shorthorn cow.

5. Subcutaneous injection of an aqueous suspension of diethylstilbestrol (1 g.) was equally successful when applied to the same cow as 4, but in the next lactation. In three Ayrshires the increase in solids was accompanied by an appreciable decline in milk yield. A Shorthorn receiving 375 mg. showed a temporary rise in solids, while one receiving 225 mg. showed no effect.

6. In all cases where milk yields declined the milk solids percentage rose, but the converse did not hold. Hence, the threshold dose for inhibition is apparently higher than for enrichment.

7. The threshold doses may depend on the breed; the most successful results were obtained with Shorthorns.

8. Treated cows may be difficult to get in calf subsequently, especially those treated twice.

9. Administration of large doses of diethylstilbestrol to cows in advanced pregnancy results in abortion.

10. The enrichment of the milk in favorable cases represented a true increase in the yield of solids secreted, and not merely a concentration due to reduced secretion of water. S.T.C.

768. Comparison of Assay Methods Using International Standard Lactogen. J. MEITES, A. J. BERGMAN AND C. W. TURNER, Univ. Missouri, Columbia. *Endocrinology*, 28: 707. 1941.

Three methods of assay of International Standard lactogen were compared, all assays based upon a 50 per cent minimum crop gland proliferation response in 20 common pigeons weighing 300 ± 40 gm. The subcutaneous route of administration required 0.1 mg. of the International Standard to equal the International Unit. The shallow intrapectoral method required 1.25 International Standard Units and the intradermal (micro) method required 1/160 of an International Unit. In connection with the intradermal method it was shown that a 2, 3 and 5 fold difference in injection volume containing the same amount of hormone caused no change in effectiveness of the crop gland responses. R.P.R.

769. Influence of Lactogenic Preparations on Production of Traumatic Placentoma in the Rat. HERBERT M. EVANS, MIRIAM E. SIMPSON AND WILLIAM R. LYONS, Dept. Anatomy, Univ. California, Berkeley, Calif. *Soc. Expt. Biol. and Med. Proc.*, 46: 586. 1941.

Experiments were conducted which demonstrated that the lactogenic hormone was the only pituitary preparation which would stimulate the production of progesterin by either normally occurring or artificially induced lutein tissue in the rat. R.P.R.

770. Local Responses of the "Sexual Skin" and Mammary Glands of Monkeys to Cutaneous Applications of Estrogen. T. L. CHAMBERLIN, W. U. GARDNER AND E. ALLEN, Yale University, New Haven, Conn. *Endocrinology*, 28: 753. 1941.

Small doses of estrogen in alcohol applied cutaneously induced local responses in the sexual skin of immature female monkeys. One to 3 gamma of estrone in alcohol daily for 8 to 12 days on one side produced a unilateral reaction while alcohol alone on the other side served as a control. Similar

treatment on one breast of young male monkeys induced considerably more growth in both nipple and glandular tissue of that side. The other mammary gland showed slight growth.

R.P.R.

771. Rumen Synthesis of the Vitamin B Complex on Natural Rations.

M. J. WEGNER, A. N. BOOTH, C. A. ELVEHJEM AND E. B. HART, Univ. Wisconsin, Madison, Wis. Soc. Expt. Biol. Med. Proc., 47: 90. 1941.

Six members of the vitamin B complex were determined in the rumen ingesta of a heifer fed a ration composed of natural feeds. In most cases higher values were found in the rumen ingesta than in the ration fed. With the exception of riboflavin, variation of the amount of urea or protein in the grain mixture of the ration had little if any effect on the vitamin content of the ingesta. The authors were of the opinion that the increase in B vitamins in the ingesta as contrasted with the ration fed was due to a synthesis and not to a concentration effect.

R.P.R.

772. Inability of Desoxycorticosterone to Maintain Lactation.

ROBERT GAUNT, Washington Square College of Arts and Science, New York Univ., New York City. Soc. Expt. Biol. and Med. Proc., 47: 28. 1941.

A study was made of the effect of desoxycorticosterone acetate on the lactation of rats adrenalectomized within 24 hours after parturition. The necessity of adrenal cortical secretions for the support of lactation in rats was confirmed. Desoxycorticosterone acetate, unlike adrenal cortical extract, was of no benefit at all in correcting this deficiency and might have further depressed lactation.

R.P.R.

773. The Interrelation of Oxidative and Glycolytic Processes as Sources of Energy for Bull Spermatozoa.

HENRY A. LARDY AND PAUL H. PHILLIPS, Univ. Wisconsin, Madison, Wis. Amer. Jour. Physiol., 133: 602-609. 1941.

In spermatozoa the energy requirement for the maintenance of their vital activity can be obtained from the oxidation of intracellular phospholipids or from glycolytic processes. When sugars are available to the spermatozoa the energy obtained from their breakdown to lactic acid lessens the demand on the intracellular lipids.

D.E.

774. Brunsterzeugung bei Schafen mit Geschlechtshormonen.

H. M. AUGUST, Office of Animal Health, Breslau. Züchtungskunde, 16, No. 2: 41-60. 1941.

In an attempt to cause sheep to come in heat in the spring so that fall lambs could be produced, 4,281 ewes in 28 flocks in German Silesia in the spring of 1938 were given intravenous injections of follicular hormone,

either alone or together with Prolan. Of the treated ewes 72.5 per cent accepted service and showed other signs of estrus and 43.1 per cent, (59.5 per cent of those which were served) produced lambs. Results appeared to be the same whether the folliculin was accompanied by Prolan or not. The methods used are not yet satisfactory for widespread practical application. The prospects for success are too uncertain, good results being obtained in some flocks but completely unsatisfactory results in others. Satisfactory explanations for these discrepancies were not found, but the problem is of enough practical importance and the present results are promising enough to deserve further investigation.

J.L.L.

MISCELLANEOUS

775. Selling and Advertising Dairy Products. 1. "Let the Taste Tell and Sell." H. D. BURBIDGE, Jersey Farms, Ltd., Vancouver, B. C. Canad. Dairy and Ice Cream Jour., 20, No. 1: 56. 1941.

Since nothing the dairy can say or write or picture about its products equals a taste of the product itself, sampling is an important sales aid to the progressive dairyman. The salesman should give the housewife a sample of all his products. Advice to the salesman is, (1) arouse interest, (2) cut down length of sales story, (3) arrange the sales story in the best possible sequence, (4) close with a bang. Let taste, not a description of taste, do your selling.

O.F.G.

776. Selling and Adveristing Dairy Products. 3. "Selling Through the Kiddies." H. D. BURBIDGE, Jersey Farms, Ltd., Vancouver, B. C. Canad. Dairy and Ice Cream Jour., 20, No. 3: 46. 1941.

The author suggested that an excellent approach to dairy sales is through children. Organize children's clubs, sports and contests, birthday clubs and write the children friendly letters, is his advice after having tried them. Other means of approach are through child talent radio programs and educational school films.

O.F.G.

777. Utilization of Skim Milk and Whey in Precooked Dried Soup. G. A. RAMSDELL AND B. H. WEBB, Div. Dairy Res. Labs., Bur. Dairy Indus., Washington, D. C. Food Res., 6, No. 3: 265. May-June, 1941.

Skim milk and whey were found to improve both the flavor and the body of spray dried soup mixtures when used in quantities up to 25 per cent of the weight of the dry mix.

F.J.D.

778. Corrosion of 18-8 Stainless Steel in Sodium Chloride Solutions. H. H. UHLIG AND M. C. MORRILL, Mass. Inst. of Tech., Cambridge, Mass. Indus. and Engin. Chem., 33, No. 7, 875-880. 1941.

A detailed study is reported of the effect of temperature, concentration,

and pH of aerated sodium chloride solutions, on the nature and rate of corrosion for 24 hour periods of 18-8 stainless steel. Corrosion increases sharply with temperature, reaching a maximum with 1 per cent to 10 per cent NaCl solutions at 90° C. and above but at the boiling point corrosion decreases to nearly zero due to lack of dissolved oxygen. Maximum corrosion occurred at 90° C. with 4 per cent NaCl solution. Maximum pit penetration in 4 per cent NaCl at 90° C. was at pH 6 to 7 and this fell to a minimum at pH 2.9 to 4.5 with a sharp increase in corrosion below pH 2.9. There was a drop in corrosion above pH 7 to a minimum at pH 12. B.H.W.

779. **Fleet Maintenance.** ANONYMOUS. *Milk Dealer*, 30, No. 8: 38-39, 87-90. May, 1941.

A description is given of the efficient system of fleet maintenance as carried on by the Alfar Creamery Company in West Palm Beach, Florida. To get an accurate account of what it costs to operate the trucks for the benefit of figuring cost of delivery, as well as the garage department expense, an analysis is made of each truck on separate cards. Samples of the cards used are presented.

C.J.B.

780. **An Apparatus for Comparison of Foaming Properties of Soaps and Detergents.** JOHN ROSS AND GILBERT D. MILES, Colgate Palmolive Peet Co., Jersey City, N. J. *Oil and Soap*, 18, No. 5: 99. May, 1941.

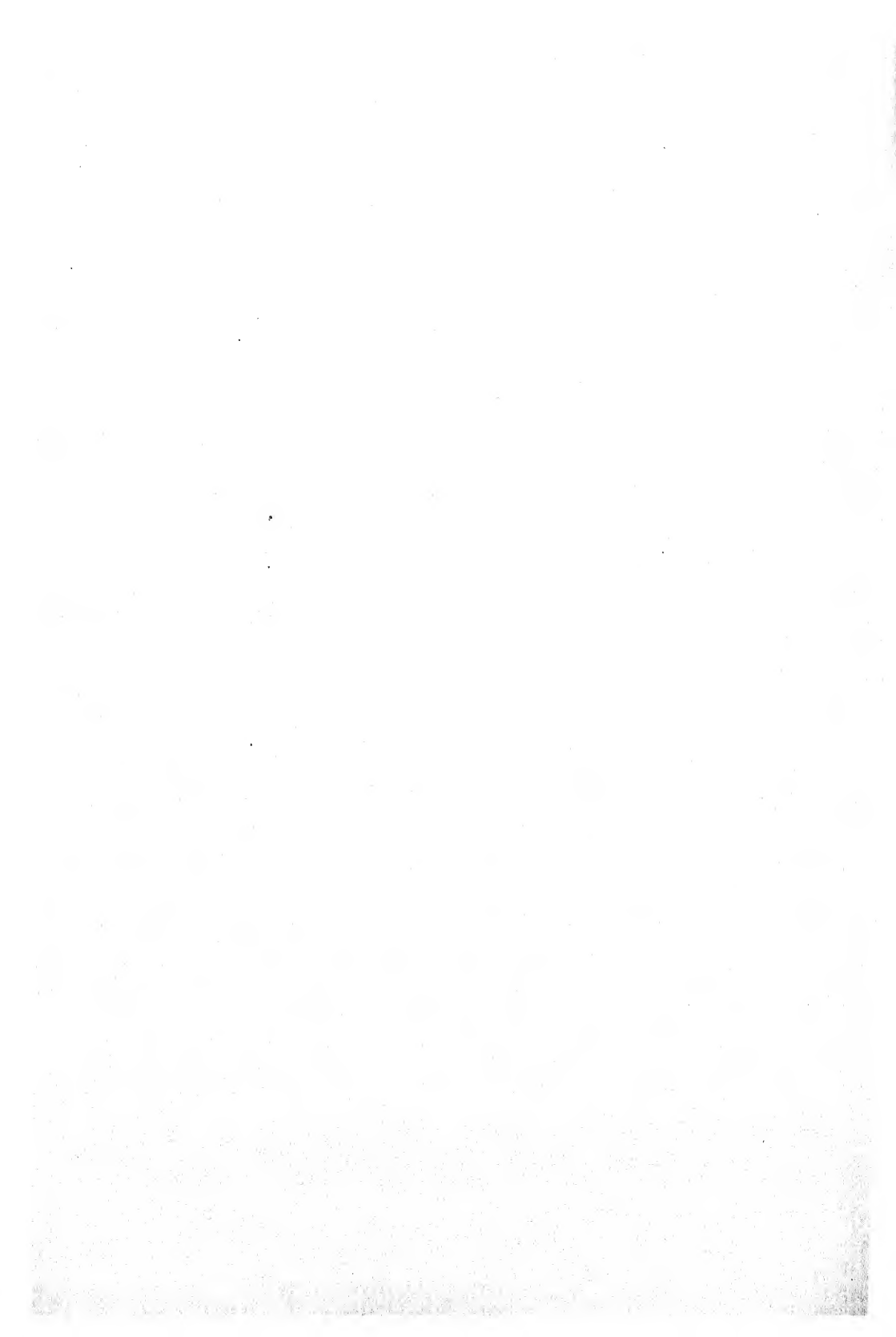
A simple apparatus and procedure is described for measuring the foaming properties of soaps and detergents. By this method the relative stability of foams is compared by measuring the effect of an arbitrary standard destructive mechanism acting upon the volume of foam during production under standard conditions and protected from adventitious destructive forces.

V.C.S.

781. **The Use of Neoprene in Refrigeration Equipment.** H. LOGAN LAWRENCE, E. I. duPont de Nemours and Co., Wilmington, Del. *Refrig. Engin.*, 41, No. 6: 404. 1941.

Details of the use of neoprene, a substitute for rubber, in small type refrigeration equipment, it being little effected by the usual refrigerants. It is an excellent rotary sealing material and is especially efficient as an insulating material for motor windings of sealed-in units even for motor sizes up to 7½ h.p. It is also used for refrigerator door gaskets and machine gaskets. While the price of a neoprene gasket is 25 per cent greater than that of a high-grade rubber gasket, the increased service life much more than offsets the higher first cost.

L.M.D.



JOURNAL OF DAIRY SCIENCE

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ABSTRACTS OF LITERATURE

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New York Association of Dairy and Milk Inspectors	United States Department of Agriculture

ABSTRACTS OF LITERATURE

ADVANCE ABSTRACTS OF REPORTS ACCEPTED FOR PUBLICATION IN THE JOURNAL OF DAIRY SCIENCE

782. **Repeatability of Type Ratings in Dairy Cattle.** LESLIE E. JOHNSON
AND JAY L. LUSH, Iowa State College, Ames, Iowa.

Ratings for type in the Iowa State College Holstein-Friesian herd according to methods intended to be like those of the official voluntary type classification and extending over an eight-year period were examined for repeatability and other characteristics. The average repeatability was .34 among ratings by experienced judges who did not know the herd. This is about the same as the usual intra-herd repeatability of production records.

Ratings made before the animals were one year old were less reliable than later ones but there was little increase in repeatability after the animals had passed one year of age.

Ratings in consecutive years were but little more alike than ratings separated by two or more years.

Changes in the udder and in the general health of the cow appeared to be the chief causes of large shifts in the rating.

783. **The Effect of Natural Milk Enzymes, Acid, and Salt upon the Keeping Quality of Butter Stored for Six Years.** B. J. SCHEIB, C. N. STARK AND E. S. GUTHRIE, New York State Dept. Agr. and Markets, Albany; and Cornell University, Ithaca, N. Y.

See JOURNAL OF DAIRY SCIENCE, 24, No. 6: 545. June, 1941, for abstract.

784. **Superheated Soft Curd Milk.** J. L. DIZIKES AND F. J. DOAN, Pennsylvania Agr. Expt. Sta., State College, Pa.

A method of producing soft curd milk by condensation, heat coagulation and homogenization, having a curd tension value of zero and digestion characteristics approximately equal to evaporated milk, is described. This product has been designated "superheated soft curd milk." When properly prepared it will exhibit digestion characteristics (*in vitro*) closely approaching those of human milk. It would require no boiling in the home and would offer fluid milk dealers, equipped to make it, a fresh milk product capable of competing with evaporated milk as far as digestibility is concerned.

785. **Various Oils and Fats as Substitutes for Butterfat in the Ration of Young Calves.** T. W. GULLICKSON, F. C. FOUNTAINE AND J. B. FITCH, Division of Dairy Husbandry, University of Minnesota.

Feeding tests were conducted to compare the feeding value for calves of the following fats and oils: butterfat, lard, tallow, coconut oil, peanut oil,

corn oil, cottonseed oil and soybean oil. The effect of a fat-poor diet also was determined.

A total of 47 young calves was used ranging in age from 6 to 29 days when placed on experiment. Their average age at start was 12 days. Each oil or fat was added to skim milk, homogenized to form a product containing 3.5 per cent fat and fed along with a low fat content concentrate mixture, cod liver oil, and usually some alfalfa hay.

One control group was fed normal whole milk, not homogenized. Test periods ranged from a few days to about six months in length.

In average daily gain in weight as well as in general appearance and well-being, the calves fed butterfat excelled those in all other groups. Following closely were those receiving lard and tallow. Corn oil, cottonseed oil and soybean oil were the least satisfactory. The average daily gains of calves in the various fat fed groups were as follows:

Butterfat	1.30 pounds
Lard	1.17 pounds
Tallow	1.24 pounds
Coconut oil96 pounds
Peanut oil80 pounds
Corn oil40 pounds
Cottonseed oil31 pounds
Soybean oil32 pounds

The calves in the three latter groups appeared unthrifty, listless and emaciated. Some calves in these groups died and others were saved only by changing to whole milk or reducing the amount of the oil fed.

Post-mortem examinations showed considerably more fat deposited in calves fed butterfat than in those receiving other oils or fats.

786. Further Studies on the Use of Salt for Improving the Quality of Cream for Buttermaking. F. E. NELSON, W. J. CAULFIELD AND W. H. MARTIN, Kansas Agr. Expt. Sta.

Laboratory and farm studies showed that the placing in the container at the beginning of seven- and ten-day collection periods of sufficient salt to give a concentration of 10 or 13 per cent in the fat-free serum of the cream at the end of the collection period resulted in marked improvement of the quality of the cream. Butter made from such cream or from cream to which the same amounts of salt had been added to the total amount of cream before a ten-day holding period at 70 or 82° F. began graded from one to five points higher than butter made from cream handled under the same conditions with no salt added. Less protein degradation (as shown by formol titration) and less development of acidity occurred in salted cream than in unsalted. The organoleptic grade on salted cream always was "sweet" or "first," whereas the grade on unsalted control cream always was at least one

grade lower than that of the corresponding salted cream and frequently was two grades lower. The salt affected the microflora of the cream both qualitatively and quantitatively. Maximum total bacterial counts of salted cream were considerably below those of unsalted cream. Acid-forming bacteria grew but little in the presence of the salt. Development of yeasts and molds appeared to be prevented completely. Large paired and clumped cocci and a few sarcina constituted the dominant flora of creams to which salt had been added. Salted cream apparently is non-corrosive to stainless steels of the types tested. Dairy metal and tinned copper were found to be subject to noticeable corrosion by salt-containing cream and probably would not be suitable materials for equipment used in collecting and processing such cream.

Addition of salt to cream offers an apparently feasible method for improving the quality of much of the butter manufactured in certain areas. Acceptance by regulatory officials and by creamerymen must be obtained before the method can be placed in the hands of the cream producer.

787. A Proposed Score Grade Method of Determining the Quality of Milk. P. A. DOWNS, University of Nebraska, Lincoln, Nebraska.

A new method of determining the quality of milk by scoring is proposed which will follow very closely the standards and ideas set forth in the Hand Book of Official United States Standards for quality of creamery butter.

This proposal is the beginning effort to unify the grading or scoring of the four (4) major products with a maximum and minimum score at a single level. As far as possible it is hoped that terms for all major dairy products can be simplified as suggested in this proposed scoring grade method of determining the quality of milk.

788. Size of the Rabbit Mammary Gland with Successive Lactations. A. A. LEWIS AND C. W. TURNER, Univ. Missouri, Columbia, Mo.

It was thought that the rabbit might illustrate the influence of mammary gland development in dairy cows as a cause of the increase in milk production with succeeding pregnancies. This increased production is greater than can be accounted for by the increase in body weight. The lateral extension of the mammary glands in succeeding lactations was compared in rabbits with lines tattooed in the skin at the lateral extent of the mammary glands early in the first lactation. In eleven succeeding lactations only one case was found in which the mammary glands on one side extended past the tattoo line. The lateral extent of the glands did not increase in the other cases.

789. The Reversibility of Oxidative Inactivation of Milk Lipase in Relation to Its Activity in Cheddar Cheese. I. HLYNKA AND E. G.

Hood, Science Service, Department of Agriculture, Ottawa, Canada.

The effect of three different reducing systems on oxidized milk lipase was studied. The lipase was inactivated by aeration alone or aeration and addition of copper. The systems were: (1) the endogenous reducing systems of deaerated milk, (2) ascorbic acid, (3) cysteine. The relation of these systems to those in cheddar cheese and of the inactivation of lipase to rancid flavour is discussed.

Portions of the same milk were aerated, aerated-deaerated (cysteine or ascorbic acid added before or after), and deaerated. The milk was incubated for 4 days at 5° C. and the extent of lipase activity was determined by titration of the fat phase.

Milk lipase was protected from oxidation by the exclusion of air while aeration and 0-8 p.p.m. Cu destroyed it. Deaeration of aerated milk restored some of the activity of oxidized lipase but deaeration of aerated milk to which Cu had been added did not. Ascorbic acid gave inconclusive results. Cysteine showed definite reversibility of oxidatively inactivated lipase with or without the addition of copper.

BACTERIOLOGY

790. Differential Staining of Living and Dead Yeast Cells. D. R. MILLS, Oregon State College, Corvallis, Ore. Food Res., 6, No. 4: 361. July-Aug., 1941.

Methylene blue, methyl green and erythrosin were found to give a sharp distinction in staining between living and dead yeast cells. A solution containing 1:10,000 methylene blue is recommended for differential staining. The authors state that plates do not give representative counts of the number of living cells in a fermenting culture but merely the number of reproducing cells, while the proposed staining method produces a rapid and accurate estimate of all viable cells.

F.J.D.

791. A Study of the Germicidal Action of Ultraviolet Light. I. Use of Ultraviolet Rays in Vegetable Hydrocoolers. F. R. SMITH AND R. L. PERRY, Univ. California, Davis, Calif. Food Res., 6, No. 4: 345. July-Aug., 1941.

The portion of this study of possible interest to the dairy industry was that wherein liquids were treated with ultra-violet light radiations from lamps submerged in the liquids. This treatment increased the germicidal effectiveness of the lamps with turbid liquids. At low temperatures, however, the activity of the light was diminished due to a decrease in the energy coming from the lamps.

F.J.D.

BOOK REVIEWS

792. **Textbook of Bacteriology.** EDWIN O. JORDAN AND WILLIAM BURROWS. Thirteenth Edition. W. B. Saunders Co., Philadelphia. 1941. 731 pages, illustrated. Price \$6.00.

The present edition of this book represents a complete revision of the previous edition. The first 11 chapters deal with general bacteriology. Two chapters are devoted to immunity and the following 23 to pathogenic microorganisms, their morphology, physiology, and various characteristics of medical significance. Additional chapters discuss the virus, virus diseases of man, bacteriophage and the Rickettsiae.

One new departure carried out in this edition is the elimination of chapters devoted to highly specialized fields such as soil, industrial and dairy bacteriology and their incorporation with other materials into a chapter on bacterial physiology. Such an approach undoubtedly will find favor with the instructor who emphasizes bacteriology from the medical standpoint and is interested in the great number of industrially important microorganisms only because of their purely scientific significance. This arrangement may limit somewhat the value of the book to those who in their teaching desire to emphasize equally the medical and industrial phases of microbiology.

This book is intended primarily for students interested in the medical aspects of bacteriology and in this respect is well written and up to date. Water and milk and other foods are considered primarily from the standpoint of their importance in transmission of disease. The subject of immunity, including recent knowledge on immunochemistry and the nature and medical significance of the filterable virus, is discussed in detail. Where subject reviews are available, the author has attempted to supply these and where such reviews are not available the discussions in the text have been expanded to briefly review them. Numerous references are supplied for statements cited in the discussions.

P.R.E.

793. **Biological Stains.** H. J. CONN. Fourth Edition. Biotech Publications, Geneva, New York. 1941. 308 pages. Price \$3.40.

The fourth edition of this book maintains the high standard set in the three previous editions. A careful revision has been made to bring all statements up to date and to correct any errors occurring in the other editions.

Thirteen new dyes are included among which are chlorazol black E, important as a general purpose stain, and the series of thiazol dyes now being employed in fluorescent microscopy.

For the information of those not familiar with earlier editions of this book it is sponsored by the Commission on Biological Stains, an independent organization affiliated with the National Research Council. Included are chapters on the history of staining, general nature and classification of dyes,

the spectro-photometric analysis of dyes and theory of staining. Six chapters are devoted to discussions of individual dyes and related groups of dyes. The discussions include chemistry of the dyes and the suitability of the dyes for various biological purposes.

Other useful information is supplied in a series of tables that list important stains, their nomenclature, synonyms, application and bibliographic references. Methods for evaluating dyes as biological stains are also described. Numerous references to original articles are provided. P.R.E.

BREEDING

794. **Achondroplasia in Calves.** GRAYDON W. BRANDT, Ohio State University, Columbus, Ohio. *Jour. Hered.*, 32, No. 6: 183-186. 1941.

The literature dealing with achondroplasia in cattle is reviewed and two cases of achondroplasia are reported which are believed to be of the recessive type. J.L.L.

BUTTER

795. **What Is Your Part in the National Quality Campaign?** H. C. DARGER, Amer. Butter Inst., Chicago. *Natl. Butter and Cheese Jour.*, 32, No. 9: 14. 1941.

Improvement of butter quality in the future will depend on cream quality which is indicated to a limited extent by tests for mold mycelia in cream and butter. Cream quality campaigns are necessary; the most effective plan is to educate creamery managers and procurement supervisors in a state-wide meeting so that they in turn can hold district meetings. Buyers meetings can be organized in the same way. This organization should be maintained by the industry. Creamery men can cooperate financially, also by grading and by observing the three principal factors in producing and marketing quality cream, namely, Sanitation, Time, and Temperature. Judicious use of the mold mycelia test can be helpful in educating patrons. W.V.P.

CHEESE

796. **Valuable Information on Process Cheese.** C. R. BARKER. *Natl. Butter and Cheese Jour.*, 32, No. 8: 14. 1941.

Uniformity of flavor and consistency are attained by intelligent blending, critical cleaning, and trimming, coarse to medium grinding, controlled heat treatment, thorough stirring and protective packaging. The base or 70 per cent of a batch is "short-held" cheese, one to three months old and "storage," six months held cheese. This cheese should be smooth and buttery, not sticky or crumbly, and two-thirds should be on the "acid-side" but not acid while one-third should be sweet. Short-held cheese tends to

make a firm, tough body while storage cheese softens the body. About 15 per cent of the batch should be "current" cheese, tough body, while 12 to 15 per cent can be "acid" cheese. The blend is most apt to be successful when cheeses from 5 to 7 factories, preferably from different sections of the United States, are used. Cheese should be tempered to 70° F. in 72 hours before blending. Emulsifiers, such as sodium citrate, di-sodium phosphate, sodium potassium tartrate, glyconates, ortho-, meta-, and poly-phosphates and di-basic citric acid salts and a caseinate are used at the rate of 1 to 2 per cent of the weight of the cheese; too much may make a stiff, coarse body. Overrun caused by the addition of emulsifier, salt and water usually equals 5 per cent for American cheese and higher for other varieties. The heating process pasteurizes and prepares the cheese for packaging. Transparent wrappers are displacing foil.

W.V.P.

797. **Factors Affecting Quality of Cheddar Cheese.** S. L. TUCKEY, Univ. Illinois, Urbana. *Natl. Butter and Cheese Jour.*, 32, No. 9: 12. 1941.

Bacterial content, acid development, moisture and salt are important factors affecting flavor of Cheddar cheese. Decomposition of the protein is of prime importance but recent experiments indicate that hydrolysis of fat and percentage of salt in the cheese also contribute to flavor development. The influence of salt was investigated by making ten lots of cheese. The curd was salted and, after 20-, 40- and 60-minute intervals, portions were removed from the vat and pressed. In the meantime the salty whey draining from the curd was poured back over the curd. Lots of cheese salted 60 minutes were superior to those salted for shorter periods because they scored higher in flavor, contained more salt, and were never criticized for bitter flavor. When the salt content was 1.7 per cent or more, ripening was delayed and the cheese body was "curdy." Prolonging the salting period beyond 60 minutes incorporated more salt and decreased the moisture. The scores indicate that cheese to be aged should contain more salt than cheese to be marketed fresh. Changes in cheese fat constants including iodine, saponification, Reichert Meissl, Polenske and acid numbers were followed for a year. The acid number showed the most significant changes indicating hydrolysis of fat. Lipase action was used to stimulate fat hydrolysis by adding homogenized raw cream to pasteurized skimmilk. A rancid flavor was produced that persisted in the cheese for at least 16 weeks. Experiments to study ripening changes are being continued.

W.V.P.

798. **Determining Volatile Acids in Cheese.** J. C. MARQUARDT AND A. C. DAHLBERG, New York Agr. Expt. Sta., Geneva, N. Y. *Natl. Butter and Cheese Jour.*, 32, No. 8: 40. 1941.

Procedure for estimating volatile acids in hard and semi-hard cheeses

have been studied and a preferred standardized method is described. A 3000-ml. round bottom flask is used for generating steam. This is connected to a 2000-ml. flask for the cheese sample and a 400-ml. Liebig condenser; all side arm and flask connections are ground glass unions. The procedure involves filling the 3000-ml. flask with 2500 ml. of distilled water. The cheese is mixed with 400 ml. of water and 25 ml. of 25 per cent H_2SO_4 solution. Distillation proceeds until twenty 100-ml. portions of distillate are collected. Size of sample is regulated so that between the fifteenth and twentieth aliquots the distillate becomes neutral to deci-normal NaOH. Sample sizes allowing "complete distillation" in the twenty aliquots are: young cheddar—200 gms.; aged cheddar—100 gms.; young semi-hard (45 per cent H_2O or more)—200 gms.; and cured semi-hard—25 gms. The total deci-normal NaOH required to neutralize the aliquots of distillate from 200 gms. of cheese is called the "volatile acid equivalent." The procedure can be used in standardizing flavor in process cheese or cheese foods and is applicable in studying changes in curing cheese. W.V.P.

CHEMISTRY

799. Promoting the Oxidation of Fats and Oils. Relative Effectiveness of Different Bands of the Visible Spectrum. GEORGE R. GREENBANK AND GEORGE E. HOLM, Bur. Dairy Indus., U. S. D. A., Washington, D. C. Indus. and Engin. Chem., Indus. Ed., 33, No. 8: 1058. 1941.

The effectiveness of different light bands in promoting oxidation of corn oil, cottonseed oil, lard and butter oil increases with a decrease in the wave lengths used, energy from the blue end of the spectrum being most effective. The light bands were obtained with the use of filters and a monochromator, and were adjusted to equal intensity. The amount of light absorbed in the yellow, orange and red regions of the spectrum was almost constant for each oil and only in lard did it exceed 5 per cent. Light absorption and amount of peroxides formed increased progressively as the wave length of light decreased from the yellow, the amount of peroxides produced being dependent upon the type of oil used. B.H.W.

800. Microbiological Assay for Pantothenic Acid. F. M. STRONG, R. E. FEENEY AND ANN EARLE, Univ. Wisconsin, Madison, Wis. Indus. and Engin. Chem., Analyt. Ed, 13, No. 8: 566. 1941.

A method for the determination of pantothenic acid has been developed which utilizes the need of *Lactobacillus casei* E for this substance. A medium essentially free from pantothenic acid but otherwise adequate for this organism is used. The lactic acid produced during growth of the organism in a medium containing the unknown is determined by direct titration

and the quantity of pantothenic acid calculated by means of a standard curve. Investigation has shown the method to be reliable. The pantothenic acid content of a number of biological materials including several dairy products is given.

B.H.W.

801. **Application of the Refractometer to Determination of Total Solids in Milk Products.** V. D. LUDINGTON AND E. W. BIRD, Iowa State College, Ames, Iowa. Food Res., 6, No. 4: 421. July-Aug., 1941.

Studies revealed that the total solids of skimmilk at various stages of condensation and dilution can be determined refractometrically with sufficient precision for commercial usage, standard deviations indicating that the measurements on which calculations are based are accurate. The use of ammonium hydroxide in the skimmilk showed no advantage.

Refractive indices of milk and cream cannot be determined with accuracy due to the dispersion of light by the fat emulsion. Satisfactory readings, however, can be obtained in samples to which 60 per cent sucrose is added but the variations in the calculated total solids are too great to make the procedure commercially feasible. No advantage was found in reading the samples at 40° C. (125° F.) as compared with 25° C. (77° F.). F.J.D.

DISEASE

802. **Sodium Chlorate Poisoning in Cattle.** G. R. MOORE, Dept. Surgery and Med., Kansas State College. Jour. Amer. Vet. Med. Assoc., 99: 50. 1941.

Sodium chlorate (NaClO_3) according to the author has been widely recommended in recent years as a means of eradicating quack grass and bindweed. The author is of the opinion that this type of poisoning in cattle and swine may be more common than is generally suspected. Causes and symptoms are described. Total dosage is discussed together with diagnosis and treatment. Prevention is suggested as the best remedy, consisting of: 1. Salt the herd thoroughly before taking any chance with them on chlorate sprayed ground. 2. Avoid using the spray in dry form, especially avoid piles on the ground. 3. Remove empty containers. F.E.

803. **Further Investigations of Rumen Gases and Bloat in Ruminants.** R. W. DOUGHERTY, Oregon Expt. Sta., Corvallis, Ore. Jour. Amer. Vet. Med. Assoc., 99: 110. 1941.

An interesting group of experiments is reported in which the H_2S present in rumen gases is correlated with the type of feed ingested. The greener and higher quality the feed the more H_2S was found in the rumen gas samples. The amount of carbon monoxide (CO) was also determined, but its occurrence was found to be similarly correlated with the type of feed in-

gested. The amount of H_2S required to produce prostration varied greatly when introduced through a rumen fistula. Operative, sampling and analyses techniques are described. F.E.

804. Studies in Brucellosis. I. F. HUDDLESON, W. B. ARDREY, R. B. PENNELL, W. H. STAHL, E. E. HAMANN, AND MYRTLE MUNGAR. Mich. Agr. Expt. Sta. Tech. Bul., 177. 1941.

This report comprises a series of nine papers. Subjects investigated were: 1. A study of factors influencing the isolation, cultivation, and differentiation of the species of *Brucella*. 2. The presence of a capsule on *Brucella* cells. 3. Study of cross skin sensitization between *Pasteurella tularensis* and *Brucella mellitensis*. 4. The immunizing value of the gluco-lipid antigen from *Brucella* cells. 5. Conditions for maximum precipitation and agglutination of antibody in various *Brucella* antisera. 6. The presence of *Brucella* antibody in the urine of human beings. 7. Separation and study of the lipid fraction of *Brucella abortus*. 8. A study of the leucocytic picture in brucellosis. 9. A study of the effect of the toxic fraction from *Brucella* cells on the leucocytic picture in normal guinea pigs.

In these studies it was shown that more than 0.5 per cent peptone in the medium tends to retard growth of *Brucella*. A concentration of 1:700,000 of crystal violet was recommended when beef liver agar containing 0.5 per cent peptone is used in isolation. Bacto-Tryptose agar was highly satisfactory for the isolation and differentiation of the three species of *Brucella* in the presence of dyes.

Data indicated that the presence and amount of capsular material determined virulence of the cells. A special procedure for capsule demonstration was described. Results suggested that when the clinical diagnosis on humans is doubtful a skin test with protein nucleate solution from *Brucella* or *P. tularensis* should serve as a means of differentiating brucellosis and tularemia. The gluco-lipid antigen from *Brucella* cells exhibited no immunizing value against experimental *Br. abortus* infection in guinea pigs.

To obtain precipitation of the maximum amount of *Brucella* antibody nitrogen for cow antiserum and endoantigen, two hours at 37° plus 22 hours at 4° C. were required. Forty-eight hours at 4° C. provided maximum removal of *Brucella agglutinin* antibody nitrogen for cow antiserum and homologous cells. *Brucella* antibody was demonstrated in the urine of certain brucellosis patients. The method for concentrating this antibody was described. The lipids which composed about five to six per cent of the cells were found to be biologically inactive.

Leucopenia with a relative lymphocytosis and slight monocytosis characterized the blood picture in *Brucella mellitensis* infected individuals. Non-filamented neutrophils were higher than normal. Pathologic lymphocytes with liver endothelial cells were found in 40 per cent of the cases. All

cases exhibited finally varying degrees of basophilic granulation of neutrophils. The data indicated that the *Brucella* toxic fraction aided by tissues of the gastro-intestinal tract had a cytolytic effect on neutrophils in the blood vessels.

P.R.E.

FEEDS AND FEEDING

805. Comparative Feeding Value of Silages Made from Napier Grass, Sorghum and Sugarcane. A. L. SHEALY, W. G. KIRK, AND R. M. CROWN. Fla. Agr. Expt. Sta. Bul. 358. 18 pages. 1941.

Three feeding trials with steers were conducted. The same amount of concentrates, consisting of ground snapped corn and cottonseed meal, was fed to each lot together with as much of the respective silage as the steers would consume. In each of the three trials more sorghum silage was consumed than of either of the other kinds.

Sorghum silage contained more dry matter, crude protein, and nitrogen-free extract than did either Napier grass silage or sugar cane silage. Due to higher digestibility of its crude fiber and N-free extract the sorghum silage contained more T.D.N. than either of the others did.

	Sorghum silage	Napier grass silage	Sugar cane silage
Average daily gains (lbs.)	2.08	1.81	1.79
T.D.N. required per lb. of gain (lbs.)	6.16	6.30	6.27
Value for fattening steers on a comparative basis	100	75	70

J.G.A.

806. Growth and Development. III. Relation between Organ Weight and Body Weight in Growing and Mature Animals. S. BRODY AND H. H. KIBLER. Mo. Agr. Expt. Sta. Res. Bul., 328. 41 pages. 1941.

This bulletin should be a valuable reference for workers in animal physiology.

Detailed charts are presented relating the weights of each of the major visceral organs to the corresponding body weights in (1) mature mammals of different species; (2) mature birds of different species; (3) animals of the same species, growing and mature. The relative-growth equation $Y = aX^b$ was fitted to each set of data and the numerical values of the exponent, b , discussed with reference to the metabolic levels. For mature animals of different species, the weights of the neuro-endocrine systems, such as the brain and pituitary gland, which are the metabolism-controlling organs, tend to increase with approximately the same fractional power as does basal metabolism; the cardio-respiratory systems, such as the heart, which carries

the working burden of the body, tends to increase more directly with body weight than with the basal metabolism. The organ-body relations in growing animals vary with the stage of growth; the value of *b* tends to be lower for later growth in the same species than for mature animals of different species. The results are discussed from theoretical and practical view points.

A list of 50 references is given.

J.G.A.

807. **Condensed Whey as a Profit Item.** J. EDWARD TUFFT. Natl. Butter and Cheese Jour., 22, No. 8: 11. 1941.

Whey condensed to 35 to 50 per cent solids and placed in 50, 500, and 600 pound drums is being sold at a profit by a California creamery to poultrymen and feed mills.

W.V.P.

808. **Distribution of "Trace Elements" in the Newborn Calf as Influenced by the Nutrition of the Dam.** L. L. RUSOFF. Fla. Agr. Expt. Sta. Bul. 359. 47 pages.

The tissues and organs of three newborn calves from normal dams and one newborn calf from a "salt sick" dam were analyzed for trace elements by a spectrographic estimation.

Of the 27 trace elements for which the various organs and tissues in this study were analyzed, 15 were detected, namely: aluminum, barium, boron, chromium, cobalt, lead, manganese, molybdenum, nickel, silver, strontium, tin, titanium, vanadium and zinc. The following elements were not detected in any of the tissues or organs: arsenic, antimony, beryllium, bismuth, cadmium, cesium, lanthanum, lithium, thorium, tungsten, yttrium, and zirconium.

Zinc was found in all of the tissues and organs which were examined; aluminum and manganese were detected in practically all of the tissues; barium, lead, molybdenum and strontium in about one-half of the tissues; nickel and silver in about one-third, and boron, chromium, tin, titanium, cobalt and vanadium were found sporadically.

A comparison of the distribution and concentration of trace elements in the tissues and organs of newborn calves from normal and from "salt sick" dams showed no significant differences.

Thirty-two references; 12 tables, 5 of which are in the appendix are given.

J.G.A.

809. **Distribution and Concentration of Copper in the Newborn Calf as Influenced by the Nutrition of the Dam.** L. L. RUSOFF. Fla. Agr. Expt. Sta. Tech. Bul. 356. 61 pages. 1941.

A comparison of the copper content of the tissues usually analyzed, of four newborn calves, as influenced by the nutrition of their dams, was made by a quantitative spectrographic method.

A comparison of the copper content of all tissues and organs of two of the newborn calves, one from a normal dam and one from a "salt sick" dam, also was made.

Copper was present in every organ and tissue analyzed.

The entire normal newborn calf contained 236.6 to 237.0 milligrams of copper and the entire "salt sick" newborn calf contained 269.9 to 271.2 milligrams.

With the exception of the skeletal tissues, practically all of the "salt sick" tissues and organs contained more copper than corresponding normal organs and tissues.

The hypothesis that bone may be a site for copper storage is supported.

The dam's being "salt sick" does not influence copper storage in the newborn calf.

No definite conclusions can be drawn concerning the role of copper in "salt sick." More biological work needs to be done before a physiological interpretation of the metabolism of copper in "salt sick" can be given.

A calf from a "salt sick" dam is not itself "salt sick."

Eighty-two references; 28 tables, 7 of which are in an appendix to the bulletin, are given.

J.G.A.

810. The Value of Urea in the Synthesis of Protein in the Paunch of the Ruminant. I. In Maintenance. L. E. HARRIS AND H. H. MITCHELL, Univ. Illinois, Urbana. Jour. Nutr., 22: 167-182. 1941.

Sheep fed a basal ration low in protein were on negative nitrogen balances. These negative nitrogen balances were always improved when either casein or urea was incorporated in the ration. The data showed that sheep may be maintained in nitrogen equilibrium for more than 100 days on rations containing urea and minimal amounts of protein providing only one-tenth of the amount of nitrogen needed for equilibrium. At nitrogen equilibrium the biological value of the urea nitrogen was 62 and of casein nitrogen 79. Urea improved the digestibility of cellulose markedly.

C.F.H.

811. The Value of Urea in the Synthesis of Protein in the Paunch of the Ruminant. II. In Growth. L. E. HARRIS AND H. H. MITCHELL, Univ. Illinois, Urbana. Jour. Nutr., 22: 183-196. 1941.

The addition of urea to a low-nitrogen ration which was in itself unable to support appreciable growth in lambs or even consistently to maintain nitrogen equilibrium converted it into a ration capable of promoting a normal or nearly normal rate of growth. Tests with casein indicated a clear superiority of casein nitrogen at levels of 15 per cent protein equivalent over the urea nitrogen at either 15 or the 11 per cent protein equivalent level. Rations containing 3.16 per cent urea on the dry basis were not toxic.

C.F.H.

812. **The Vitamin A Requirements for Normal Growth in Young Dairy Cattle.** H. T. CONVERSE AND E. B. MEIGS, U.S.D.A., Washington. Amer. Soc. Anim. Prod. Proc., 32: 67-72. 1939.

Calves were fed from the fourth day of age a low vitamin A basal ration supplemented with varying amounts of carotene ranging from 29 to 87 μ g. per kg. body weight and similar levels of vitamin A ranging from 7.55 to 22.65 μ g. per kg. body weight. On both the highest and lowest levels calves died or developed subnormally, and the results indicated that the optimum was an amount approximately twice the lower level. At this age the requirement was larger per unit of body weight than for ages 6 to 24 months.

G.C.W.

813. **Carotene Requirements of Dairy Cattle for Reproduction.** A. H. KUHLMAN AND W. D. GALLUP, Oklahoma A. and M. College, Stillwater. Amer. Soc. Anim. Prod. Proc., 33: 67-73. 1940.

Using a basal ration of cottonseed meal and prairie hay it was found that a minimum of 40-45 μ g. of carotene per pound of body weight was required daily by Jersey cows during the 270 days preceding parturition to produce normal calves, and to start a normal lactation.

G.C.W.

814. **High-phosphorus vs. Low-phosphorus Red Clover Hay for Growing Calves.** H. R. DUNCAN, Univ. Tennessee, Knoxville. Amer. Soc. Anim. Prod. Proc., 33: 109-111. 1940.

The only difference in the ration of calves each receiving 6 lbs. of clover hay, ground yellow corn, salt and cod liver oil was the P content of the hay, being 0.116 per cent and 0.309 per cent, respectively. In 196 days the average gain in weight was 79 lbs., in height at withers 2.21 inches, and in heart girth 2.46 inches for the low P group compared to 124 lbs., 3.46 inches and 5.42 inches, respectively, for the high P group. The cost of a given amount of gain was 33 per cent more for the low P fed animals, and they were decidedly less thrifty. The low P hay was grown under circumstances assumed to be favorable on a farm much above the average in productiveness.

G.C.W.

815. **Further Studies of the Influence of Fat Intake on Milk and Fat Secretion.** L. A. MAYNARD, J. K. LOOSLI AND C. M. McCAY, Cornell Univ., Ithaca, New York. Amer. Soc. Anim. Prod. Proc., 33: 340-344. 1940.

Two recent trials, one continuous and one double reversal, comparing grain rations of 7 per cent and 3 per cent fat, respectively, bear out earlier results in this laboratory of increased milk and fat percentage yields on the higher fat rations. The several trials (10) show increased daily yields of

fat-corrected milk ranging from 0.27 to 3.54 lbs. per cow. It is not known whether fat *per se*, or some fatty acid, or some other component of ether extract has been responsible. G.C.W.

FOOD VALUE OF DAIRY PRODUCTS

816. **The Effect of Certain Fats and Unsaturated Fatty Acids upon the Utilization of Carotene.** W. C. SHERMAN, Lab. of Anim. Nutr., Ala. Agr. Expt. Sta., Auburn. Jour. Nutr., 22: 153-165. 1941.

The effect of supplementing a diet low in fat and carotene with linseed oil, corn oil, wheat germ oil, butterfat, cottonseed oil, methyl linolate and methyl linolenate on carotene utilization was investigated with rats. Soybean oil, cottonseed oil, linseed oil, corn oil, and wheat germ oil had a beneficial effect upon growth. Butterfat and coconut oil had no appreciable effect. Methyl linolate and methyl linolenate had an antagonistic action when fed with low levels of carotene which was counteracted by the addition of soybean oil and also by feeding carotene and methyl linolate a few hours apart. C.F.H.

817. **Butter and Nutrition.** ALICE COOLEY, Natl. Dairy Council, Chicago. Natl. Butter and Cheese Jour., 32, No. 9: 16. 1941.

There are three basic reasons why a home-maker should buy butter. First, it is a dependable source of vitamin A since it is eaten three times daily and because the vitamin A in butter is better utilized than carotene, the form in which the vitamin occurs in fruits and vegetables. Vitamin A builds resistance to infection and a deficiency of it causes "night-blindness" and eventually xerophthalmia. Second, butter contains a growth-promoting factor other than vitamin A. Data show the superiority for growth in young animals of butterfat over such vegetable oils as corn, cotton seed, soy bean and cocoanut although the known fat soluble vitamins, such as A, D, and E, were adequately provided. Third, our people use less than half as much butter as other English-speaking people, yet there is no substitute for butter. Flavor and digestibility of butterfat should commend its use. Fortification of other foods with vitamins requires more research to disclose the importance of butter in the diet. W.V.P.

818. **Further Experiments on the Calcium Requirement of Adult Man and the Utilization of the Calcium in Milk.** F. R. STEGGERDA AND H. H. MITCHELL, Univ. Illinois, Urbana. Jour. Nutr., 21: 577-588. 1941.

Twenty-five calcium-balance periods of 12 to 32 days each were carried out on nine adult men. The basal diet which furnished 203 mg. of calcium daily was supplemented with milk products (liquid whole milk, liquid skim

milk, "dried milk solids" and homogenized milk) in amounts to provide enough calcium for approximate equilibrium. The calcium in the different milk products studied was utilized at about the same degree of efficiency. An average utilization of 29 per cent was observed. The average calcium requirement for equilibrium was 9.55 ± 0.46 mg. daily per kilogram of body weight. C.F.H.

819. **Pasteurization and the Nutritive Value of Milk.** C. A. ELVEHJEM, Univ. Wisconsin, Madison, Wis. *Dairy World*, 20, No. 3: 18. Aug., 1941.

This paper is a very good summary of recent studies and recent thought relative to the effect of heat on the nutritive properties of milk. The author does not confine himself merely to comparisons of pasteurized and raw milk but touches on seasonal variations, higher heat treatments, exclusive feeding of milk to animals through more than one generation and several other interesting related subjects. In conclusion he states that, "we are unable to find on a nutritional basis any objection to the production and use of pasteurized milk." F.J.D.

820. **The Ascorbic Acid Content of Cows' Milk during Pregnancy.** A. D. HOLMES, F. TRIPP, E. A. WOELFFER AND G. H. SATTERFIELD, E. L. Patch Co.; H. P. Hood and Sons, Boston, Mass., and Univ. North Carolina, Raleigh. *Jour. Nutr.*, 22: 267-271. 1941.

The ascorbic acid content of the milk of Guernsey and Holstein cows was determined from time to time throughout pregnancy. The animals were stall fed. The ascorbic acid values showed considerable variation although they tended to decrease slightly with advanced pregnancy. C.F.H.

821. **Nutritive Value of Quick Frozen Foods.** FAITH FENTON, Cornell Univ. *Refriger. Engin.*, 42, No. 2: 140. 1941.

The vitamin C content of quick frozen vegetables was studied. In general, it was found that the vitamin C content was very high in quick frozen vegetables and much higher than that of fresh vegetables which have been held even a few hours at room temperature. The author cautioned against the use of too much water in cooking the vegetables and then discarding it because of the high degree of solubility of the vitamin. For the same reason frozen vegetables should not be thawed before cooking and they should be cooked in only a very minimum of water. L.M.D.

ICE CREAM

822. **A Study of Skim Milk for Ice Cream.** L. K. CROWE AND HARRY H. WINN, Univ. Nebraska. *Ice Cream Field*, 38, No. 2: 40. 1941.

The authors discuss the results obtained in an investigation designed to

study the effects of freezing and storing upon (a) plain condensed skim milk, (b) superheated condensed skim milk and (c) sweetened condensed skim milk. A review is given of previously published work related to the problem.

It was found that fresh plain and sweetened condensed skim milk were almost completely soluble and only very slightly more soluble than fresh superheated condensed skim milk. The solubility of the plain condensed skim milk decreased slowly over the three months storage period, whereas the solubility of the superheated condensed skim milk decreased rapidly over this same period. The sweetened condensed skim milk was almost completely soluble after three months storage.

The results showed no consistent change in protein stability (alcohol number) of ice cream mixes made with condensed skim milk which had been stored frozen for one month when compared to mixes prepared with fresh condensed skim milk. It is stated "Mixes made with stored frozen superheated condensed skim milk showed less change than mixes prepared with plain or sweetened condensed skim milk." Protein stability of ice cream mixes made with each type of condensed skim milk stored frozen two months was greater than that of mixes made with fresh skim milk or condensed skim milk stored frozen for one or three months. No apparent relationship was found between the solubility of condensed skim milk and the alcohol number of ice cream mixes in which it is used.

There was very little difference in the titratable acidity of the ice cream mixes made with the three types of fresh condensed and the results indicated that freezing and storing condensed skim milk for periods up to three months did not increase the titratable acidity of ice cream mixes in which it was used. The pH of the ice cream mixes was always within the range 6.2 to 6.4.

Viscosity of ice cream mixes was not consistently affected by the freezing and storing of the three types of condensed skim milk used. Mixes made with fresh plain condensed skim milk whipped to 100 per cent overrun faster than ice cream mixes made with fresh superheated and fresh sweetened condensed skim milk. Mixes made with the three types of condensed skim milk stored frozen for one month whipped more slowly than those prepared from the corresponding fresh condensed skim milks. It is stated, however, that the time required to reach 100 per cent overrun in ice cream mixes made with the three types of condensed skim milk stored frozen was less than the time required for mixes made with fresh condensed skim milk.

There was no appreciable difference in the flavor scores of ice cream attributed to the type of condensed skim milk used whether fresh or frozen, but there was a tendency for the body and texture scores to be less as a result of using the three types of frozen condensed skim milk. Freezing and storing plain and superheated condensed skim milk increased the amount of foam, whereas the opposite was true for sweetened condensed skim milk.

Ice creams made with the frozen condensed skim milk were frequently criticized for flaky or curdy appearance upon melting. W.C.C.

823. **Effects of Freezing and Hardening on Ice Cream Texture.** J. H. ERB, Ohio State Univ., Columbus, Ohio. *Ice Cream Field*, 38, No. 2: 46. 1941.

The author describes a laboratory experiment carried out in connection with a class in ice cream in which a given ice cream mix was frozen and hardened under four different conditions. The results showed that the body and texture scores varied as much as 2.5 points. The poorest ice cream was produced by drawing it from the batch freezer in a soft condition and hardening it slowly at 0° F. Ice cream taken from the same freezer and hardened more rapidly at -30° F. showed an improved score of from 0.5 to 1.0 point. Stiff ice cream drawn from the batch freezer and hardened slowly was about equal in texture to the soft drawn ice cream which was hardened rapidly. The ice cream drawn from the continuous freezer at the same temperature as from the batch freezer showed a slightly better texture. The smoothest textured ice cream was that drawn from the continuous freezer at 22° F. and hardened rapidly. W.C.C.

824. **Ice Cream and Problem of Rising Costs.** JACK NISBET, Secretary, Ohio Assoc. Ice Cream Mfrs. *Ice Cream Field*, 38, No. 2: 20. 1941.

It is pointed out that increased costs of ice cream ingredients have necessitated some increase in the wholesale price of ice cream. In some instances this has been used as a basis of relatively large increases in retail prices resulting in marked decreased consumption. It is suggested that it may be necessary to prepare suitable streamers and signs for "6¢ cones" and "12¢ sodas" to keep the retailer from increasing his price in 5¢ jumps.

The author states that the food value of ice cream should be stressed during the present emergency to avoid having people consider it a luxury. The advisability of correcting misconceptions is emphasized and several "mistaken ideas about ice cream" are cited. W.C.C.

825. **Merchandising Ice Cream and Associated Products.** CHARLES STICH, Kats and Besthoff, New Orleans, La. *Ice Cream Field*, 38, No. 2: 28. 1941.

Attention is called to the fact that in 1931 Drug Stores accounted for 30.5 per cent of the nation's ice cream volume, whereas in 1938 it accounted for 28.6 per cent, while company-owned retail stores in 1931 accounted for 2.3 per cent of the national ice cream volume as compared with 5.7 per cent in 1938.

The author considers ice cream quality of prime importance and concludes that ice cream should have a high butterfat content, be rich, smooth and creamy and full of flavor. In addition it should have a descriptive name such as: velvet; all-cream; tru-flavor; flavor-fresh, etc.

Reference is made to a survey which shows that ice cream's greatest appeal—20 per cent—is quality, and its least appeal—2.3 per cent—is price. Ice cream appeals as a food to 15 per cent of the customers and as a refreshment to 12 per cent of them. He emphasizes the advantage of selling ice cream as a food as well as supplying suitable specials for various occasions throughout the year. Mention is also made of window displays as a means of merchandising ice cream.

The importance of "The carry-out ice cream business" is stressed and the increased sales resulting from the use of a special package for this purpose are cited.

W.C.C.

826. Are There Profits in Pecan Ice Cream? EDWARD G. MARSH, R. E. Funsten Co., St. Louis, Mo. Ice Cream Field, 37, No. 6: 26. 1941.

The author quotes the International Association of Ice Cream Manufacturers as showing by a survey that butter pecan accounts for 3.67 per cent of the U. S. ice cream production. He claims that if properly processed, pecans do not become soggy or woody when incorporated in ice cream.

Reference is also made to U. S. Department of Agriculture Bulletin 324 entitled, "An Economic Study of the Pecan Industry," which reports that if price is not a factor that consumer preference for pecans outranks their preference for all other nuts.

Shelled pecans are obtained primarily from the wild or seedling variety, whereas the papershell or improved variety is usually sold in the shell. It is stated that the pecan industry has 17 grades of shelled pecans from which the ice cream industry may select.

Various methods of introducing and merchandising pecan flavored ice creams are mentioned.

W.C.C.

827. Do You Sell a 10¢ Pint? ANONYMOUS. Ice Cream Field, 37, No. 6: 6. 1941.

This is a report of the findings of a survey by the Institute of Ice Cream Opinion which is under the direction of the Ice Cream Field. The results show that 86.9 per cent answered this question in the negative while 13.1 per cent of the replies indicated that their competitors sell a 10¢ pint. It was further revealed that 86.2 per cent of the replies favor a low priced package ice cream as a means of increasing gallonage and that the average of the price range given by these replies was 18.009¢.

There was considerable variation in different sections of the United States, with California and the South West areas showing a greater tendency to sell this low priced item than the Northern areas.

W.C.C.

828. Getting the Most out of Flavors in Ice Cream. C. A. IVERSON, Iowa State College. Ice Cream Field, 38, No. 1: 49. 1941.

It is stated that although the trend in the ice cream industry is towards more delicate flavors and colors and towards smoother textured ice cream that sudden changes even in the direction of the trends may cause consumer objection.

The author points out that serum solids have a depressing effect upon flavor in addition to any "off" flavors which they may carry. It is postulated that butterfat acts as a flavor carrier whereas serum solids to a certain extent act as flavor depressors.

In discussing "vacreation," a system of vacuum pasteurization, it is stated that from $\frac{1}{4}$ to $\frac{1}{3}$ less flavor is required to obtain approximately the same flavor intensity with vacreated mix as with mix pasteurized in the usual manner.

It is stated that it is not always possible to predict whether or not a given substance may serve as a satisfactory flavor in ice cream. It is actually necessary to try the combination in ice cream. It is suggested that caramel and fudge flavors be used in combination with chocolate and furthermore that mint flavor mixes well with chocolate.

Fresh ground coconut meat makes a very pleasing flavored ice cream. When used alone from 4 to 6 quarts of ground coconut is required for 10 gallons of finished ice cream. Coconut pineapple and coconut cherry also are pleasing combinations, it is stated.

Nuts for ice cream must have pleasing flavor and be crisp and tender in the ice cream. It is reported that dry roasting the nuts and treating them with butter previously heated to 330° F. is most satisfactory because it develops a roasted nut flavor, dries out the nut and the butter coating causes the nut to retain the crispness to a remarkable degree.

Butter is used at the rate of 1 to 2 pounds for 8 pounds of nuts.

Mention is also made of the importance of sanitation in handling and preparation of nuts. The heat treatment mentioned is a safeguard in this connection.

Pecans, English walnuts, black walnuts, almonds, and Brazil nuts are mentioned as satisfactory either alone or in various combinations. In addition hazelnuts or filberts, cashew nuts and pistachio nuts are mentioned as good possibilities.

W.C.C.

829. How Can We Improve Our Sherbets and Ices? P. H. TRACY, Univ. Illinois. Ice Cream Field, 37, No. 6: 36. 1941.

The author defines ices and sherbets according to the standard adopted by the International Association of Ice Cream Manufacturers and points out that in certain instances they play a relatively important role in the ice cream industry.

A list of seven desirable features of stabilizers is given for ices and sherbets. This is followed by a brief discussion of the more common stabilizers used including gelatin, various gums, pectin, and sodium alginate. The advantages and disadvantages of each stabilizer mentioned is briefly outlined and directions for its use are given.

The author recommends the use of monosaccharides—such as honey, dextrose or sweetose—in conjunction with cane or beet sugar to prevent surface crustation. It is stated that satisfactory results were obtained using 14 per cent sucrose and 21 per cent sweetose. The author also recommends from 0.35 per cent to 0.50 per cent citric acid content depending upon the sugar content of the mix.

Brief mention is also made of the following defects in ices and sherbets: bleeding, surface crustation, crumbly body, snowy body, coarse body, sticky body and ice separation.

A few suggested formulas are given.

W.C.C.

830. Ice Cream Sales Soar. ANONYMOUS. Ice Cream Field, 38, No. 2: 21. 1941.

A brief discussion is given of the statistical report of the United States Agricultural Marketing Service, and the Ice Cream Sales Index compiled by the Statistical and Accounting Bureau of the International Association of Ice Cream Manufacturers. It is pointed out that except for the second four-months period of 1936, the first four months of 1941 had the largest sales increase of any four-months period during the 16 years the Sales Index has been published.

W.C.C.

831. Coffee as a Summer Flavor. ALEXANDER KATZ, Florasynth Labs., Pacific Coast Div. Ice Cream Field, 38, No. 2: 34. 1941.

In a comparison of twelve flavors, coffee was selected as the outstanding flavor by the public and vanilla was considered second.

Extreme care is necessary to properly prepare concentrated liquid coffee flavor. It is stated that synthetic coffee flavor cannot replace the flavor prepared from natural coffee.

Many uses are mentioned for this product including the flavoring of ice cream, milk, milk shakes, candy, etc.

W.C.C.

832. Fruits on the March to Greater Sales. VINCENT M. RABUFFO, Editor, Ice Cream Trade Jour., New York. Ice Cream Trade Jour., 37, No. 7: 15. 1941.

The sale of fruit flavored ice cream has been increasing. Black and red raspberries, cherries, pineapples, and peaches are being used in ever increasing quantities. A knowledge of the characteristics of the various varieties

of fruits, how to pack and preserve them for ice cream, and the taste and eye appeal which fruit impart to ice cream, are factors responsible for their increased use. Amounts used, common varieties, sources and methods of preparation for use in ice cream are discussed in this article. W.H.M.

833. **Profits the Milky Way.** LOUIS D. JONES, Hamilton Beach Co., Racine, Wis. *Ice Cream Trade Jour.*, 37, No. 7: 12. 1941.

A description is given of how a successful merchandiser boosted his sale of ice cream by featuring ice cream and milk drinks. A creamy delicious malted milk or milk shake, an ice cream soda, tangy-zestful with flavor varieties to create wide taste appeal and a sundae with outstanding eye and taste appeal were used as leads. High quality ingredients, properly trained help, personal interest in his customers and accurate cost records are listed as some of the major points in his success. W.H.M.

834. **Current Sales Trends.** O'NEAL M. JOHNSTON, Washington, D. C. *Ice Cream Trade Jour.*, 37, No. 6: 39. 1941.

With the exception of January, May, August and September, every month in 1940 showed an increase in the sale of ice cream in the United States according to figures released by the Statistical and Accounting Bureau of the I. A. of I. C. Manufacturers, Washington, D. C. in Special Bulletin Number 65. The net increase for the year was 2.56 per cent above 1939 sales. W.H.M.

835. **Stabilizing Water Ices and Sherbets.** B. I. MASURVOSKY, Editor, *Ice Cream Trade Jour.* *Ice Cream Trade Jour.*, 37, No. 6: 35. 1941.

A replacement of 25 to 30 per cent of the sucrose in sherbets and ices with invert sugar, dextrose or corn sirup solids is suggested. There are several stabilizers for sherbets and ices and usually satisfactory results can be obtained by following the directions furnished with these products. Poor results sometimes occur because the composition of the mix is altered when fruits are added to the mixture. W.H.M.

836. **Smoothness in Ice Cream.** G. C. NORTH, Beatrice Creamery Co. *Ice Cream Trade Jour.*, 37, No. 6: 13. 1941.

Several factors, homogenization, proper balance of ingredients and faster freezing, have tended to eliminate coarseness in ice cream texture and a smoother product has resulted. Smoothness in texture is definitely related to size of ice crystals, size and number of air cells, presence of lactose crystals and butterfat particles. Homogenization increases the total fat globule surface and produces a lubricating effect making a given sample of ice cream appear to be smoother and the fat also offers mechanical obstruction to the formation of ice crystals.

By increasing the non-fat milk solids in the mix, the formation of large ice crystals is obstructed and the amount of water available for ice formation is reduced. The use of an excessive amount of total solids should be avoided, since freezing and storage difficulties may be encountered and defects such as sandiness and soggyiness may result. A proper relationship between the fat and non-fat milk solids should be maintained and the kind and amount of sugar regulated to avoid excessive sweetness and freezing difficulties. Stabilizers and eggs also tend to improve the texture of ice cream.

W.H.M.

837. England's Ice Cream Industry under Fire. CLIFFORD SKERTCHLY, London, Eng. *Ice Cream Trade Jour.*, 37, No. 6: 10. 1941.

With the use of fresh milk and cream forbidden and the use of butter, concentrated milk products and sugar greatly restricted, ice cream manufacturers in England have turned to the use of substitute products. At first about the only change in the mix was the substitution of margarine for butterfat, later soya bean flour, corn flour and wheat flour replaced the milk solids. It also became necessary to reduce the sugar content from 13 per cent to 11 per cent and to substitute honey for part of the sugar. The author states that England's war-time ice cream seems to be palatable and its selling price shows no appreciable increase. The following is one of the recommended formulae used in England: 1 pound of fat; 8 ounces of wheat flour; 1 pound of sugar; 2 ounces of egg yolk; water to make 1 gallon.

W.H.M.

838. A New Quick Freezing System. L. H. BARTLETT AND H. E. BROWN, Bur. Engin. Res., Univ. Texas. *Refrig. Engin.*, 42, No. 2: 83. 1941.

The advantages and disadvantages of fluid contact freezing are described and the use of a polyphase freezing medium in order to overcome disadvantages of the liquid medium is explained.

This polyphase medium is a chilled solution of a freezing point depressing solute, through which is dispersed the solid phase of the solute in finely subdivided form. The mechanism of heat transfer is one largely of conduction. Decrease in the fluid film resistance of a polyphase medium is probably due to the fact that the thermal conductivity of ice is very much greater than that of the liquid phase, it being 1.3 btu. per ft. hr. °F. at 0° F., while that of a 63 per cent syrup is only 0.19 btu. per ft. hr. °F. at the same temperature. The weighted mean conductivity of a polyphase medium containing 5 per cent by weight of suspended ice in 63 per cent syrup is 0.27 btu. per ft. hr. °F., which is 1.38 times the conductivity of the liquid phase.

The polyphase medium removes a given quantity of heat from foods in

about 60 per cent of the time required by a liquid medium of similar composition and at the same temperature.

The heat transfer rates are nearly independent of fluid velocities enabling very slow circulation rates to be used, thus reducing energy losses to the medium. Also very viscous media can be employed at low temperature.

The authors have developed a continuous freezing machine of simple design employing the polyphase medium of water-invert sugar refrigerated at -2° F. capable of freezing 50 lb. of product per hour, weighing 700 lb. and requiring only 8 ft. by 16 in. of floor space. The food product is fed into a hopper at one end. The circulation is maintained by the helicoid conveyor, and by varying its speed, contact-time may be varied from 5 to 17 min. Low power is required, less than $\frac{1}{4}$ h.p. being needed (exclusive of refrigeration) to operate the 50 lb. unit. Foodstuffs are frozen as individual pieces and thus may be stored in bulk containers from which they may be packed in smaller units as desired later. Because of the elasticity of the freezing fluid, delicate food products are not broken or crushed, while agitation is secured not only by means of the spiral motion in the helicoid conveyor but by means of a unique drive gear which rotates the screw about one-fifth turn forward and then about one-sixth turn backward. L.M.D.

MILK

839. **The Filter Disc as a Sediment Tester on the Farm.** JOSEPH BURNS, Schwartz Mfg. Co., Two Rivers, Wis. Natl. Butter and Cheese Jour., 32, No. 9: 56. 1941.

It is suggested that the filter disc viewed as a sediment test by the farmer can stimulate the production of better milk. Filter discs and cellophane-covered cards on which to mount them were given to 8 farmers with the request that they return them at the end of a week to the plant receiving their milk. Plant technicians made daily methylene blue and sediment tests of each patron's milk. Inspection of the filter discs indicated that some patrons tried to keep the milk cleaner and the author believes that keeping dirt out of milk leads to other good practices. The tabulated results of the plant technicians' tests show no improvement in methylene blue or sediment during the six-day observation period. W.V.P.

840. **Proposed A.S.R.E. Methods of Rating and Testing Complete Can-type Milk Coolers.** The American Society of Refrigerating Engineers, New York, N. Y. Refrig. Engin., 42, No. 3. 1941.

Methods of rating can-type milk coolers developed by a special committee of the American Society of Refrigerating Engineers. The general purpose of this code is to provide the purchaser with the standard ratings so that he may select adequate equipment insofar as capacity and speed of cooling are

concerned. These methods, it is believed, will result in rating information entirely understandable by the dairyman. The test procedures include only electrically driven mechanical units. The methods proposed consist of Definitions, Types and Accuracy of Instruments, Rating Tests, Calculation of Test Results, General Data to be Recorded, and Test Report. The two most important ratings to be determined for any cooler are the Rated A.S.R.E. capacity and Rated A.S.R.E. power consumption. Different testing procedures are employed for non-ice-making coolers and ice-making coolers. Formulas are given for calculation of the above ratings and for the A.S.R.E. Rated Milk Cooling Coefficient, the latter being watt hours per gallon per degree F. The committee recommends that the Rated A.S.R.E. capacity and power consumption be included as one point in the manufacturers' published capacity data.

L.M.D.

841. Refrigeration Requirements in the Processing and Marketing of Milk and Milk Products. KENNETH M. RENNER, Texas Technol. College. *Refrig. Engin.* 42, No. 2: 90. 1941.

A resume of literature dealing with refrigeration requirements in connection with milk production, market milk handling, and milk products manufacture. The author points out that the following factors should be considered in their effect on refrigeration requirements: 1. The type of product that is to be refrigerated, and the specific requirements with respect to temperature control of the product. 2. The methods employed in the processing of the product. 3. The peak load in any one day's operation. 4. Type of equipment being used in the plant. 5. Methods of marketing the product.

L.M.D.

842. Marketing of Milk Products in Lenawee County, Michigan. ORION ULREY, Mich. State College Agr. Expt. Sta., East Lansing, in cooperation with the Bur. of Agr. Econ., U. S. D. A. *Spec. Bul.*, 310. June, 1941.

This is a 42-page report of one segment of a study made in various states by the agricultural experiment stations in cooperation with the United States Bureau of Agricultural Economics. This report deals with the marketing of milk and butterfat in Lenawee County, Michigan, with special consideration being given to local and interregional competition. Production conditions also were studied on selected farms in two townships.

Main purposes of the investigation were to determine the types of local market outlets, the place of the county dairy plants in processing milk products, and the competitive position of the cream from this area in the eastern markets. Special attention was given to the inspection of cream by the city of Detroit and by the cities in the East.

An attempt was made to analyze the principal milk marketing problems of the area. One of the chief problems was found to be the lack of a uniform system of inspection of farms and plants by states and municipalities and a large variation in strictness of the enforcement of regulations. P.H.T.

843. **Stability of Homogenized Milk in Cookery Practice.** HERBERT HOLLENDER AND K. G. WECKEL, Univ. Wisconsin, Madison, Wis. Food Res., 6, No. 4: 335. July-Aug., 1941.

Homogenized milk was found to be definitely less stable (more easily coagulated) in cookery practice than unhomogenized milk. In the preparation of escalloped potatoes the usual methods of stabilizing milk by adding sodium citrate, phosphate or carbonate produced variable results depending on the characteristics of the potatoes used. The acidity of the potatoes appeared not to be a measure of their effect.

In the preparation of cooked oatmeals or wheat cereals, homogenized milk produced a whiter and more creamy dish than did unhomogenized milk and the coagulation was either retarded or prevented by the use of table salt in amounts normally used for seasoning. Preheating the milk did not influence its stability in the cereals. The type and degree of water hardness also was found to be a factor influencing stability.

Homogenized milk used in egg custard caused a lower curd tension and greater serum separation; however, the use of a lower cooking temperature and a shorter period of time overcame the serum separation to a considerable degree. F.J.D.

844. **Dairy Plant Refrigerating Systems.** RAY B. WOLF, Borden Co., San Francisco, Calif. Ice and Refrig., 99, No. 4: 234. 1940.

The importance of refrigeration to the fluid milk industry is pointed out by showing that out of a total milk production of 12,500,000,000 gallons in the United States, 3,875,000,000 gallons are used for fluid milk and cream of which amount nearly 45 per cent or 1,743,750,000 gallons are pasteurized. It takes 5.35 tons refrigeration to properly handle 1000 gallons of pasteurized milk, and only about one-half of this is supplied by water. About 12 per cent of the cooling load is before pasteurization, 43 per cent after pasteurization and the balance is used for storage rooms. In addition, $\frac{3}{4}$ to $1\frac{1}{4}$ tons of ice (the equivalent of $1\frac{1}{2}$ tons refrigeration) are needed for every 1000 gallons of product for delivery purposes.

The author points out that with the exception of very large plants, it is more profitable for a dairy to buy its ice than to manufacture it. The disadvantages of brine as a cooling media in modern dairy equipment are pointed out. Chilled water, and the flooded type of direct expansion are to be preferred. The size of the unit depends on the operating procedure,

but a 6 × 6 unit will very nicely take care of a 50-quart filter. It is advisable to have auxiliary refrigerating equipment in order to avoid costly shut downs if a break down occurs.

Different types of pasteurizing machinery are briefly touched upon. Proper controls are emphasized.

L.C.T.

PHYSIOLOGY

845. Variations in Dairy Bull Semen with Respect to Its Use in Artificial Insemination. H. A. HERMAN AND E. W. SWANSON. Mo. Agr. Expt. Sta. Res. Bul. 326. 82 pages. 1941.

A comprehensive study, undertaken in order to determine the quality of semen produced by bulls whose breeding efficiency was known, to compare the actual efficiency of semen used in artificial insemination with the results of physical and chemical examinations of the sample, and to determine, if possible, the best measure of evaluation of semen to be used for artificial insemination of dairy cows under practical field conditions.

A critical examination of semen from 342 separate ejaculates representing 55 dairy bulls was made. The fertility of 51 of the bulls was determined from their breeding records and compared with the examination of their semen.

The semen samples were found to vary widely in all properties studied. The variation was observed in different ejaculates of the same bull as well as in ejaculates of different bulls. The variations in initial motility and pH were not great. The greatest variations observed were in the length of time vigorous motility persisted and percentage of abnormal spermatozoa.

The time of survival with good motility was found to be a good index of fertility of the semen. The longer vigorous motility was maintained in a sample of semen, the greater was the chance of that semen producing conception.

The pH, initial motility, and percentage of abnormal spermatozoa were found to be related to fertility of the semen to only a limited extent. Semen of high pH, 7.00 or higher, was usually of very low viability. Semen of poor initial motility survived only a short time. A normal pH, good initial motility and low percentage of abnormal spermatozoa were an indication but not assurance of good fertility.

Morphologically abnormal spermatozoa were found in every sample of semen examined. Bulls which produced semen averaging 30 per cent of abnormal spermatozoa were usually of poor fertility, but not all samples of semen containing over 30 per cent abnormal spermatozoa were infertile. The most common types of abnormal spermatozoa, in order of their occurrence, were coiled tails, tailless, and pyriform. In the semen observed in this study no one of the three main types of abnormal spermatozoa seemed of more significance to infertility than other types.

Survival time of semen which was normally of good quality was not increased by dilution with any of the common diluents used. Survival of semen from a few bulls was materially increased by dilution with egg-yolk-buffer dilutor. Such semen normally became thick and viscous in storage and the dilutor apparently prevented this or other deleterious effects.

Fertility of good quality semen was maintained from 3 to 5 days when stored undiluted at 40° F.

The fertility of a bull cannot be accurately estimated from a single semen examination. Three or more semen samples examined several days, or even weeks apart, with accompanying records of the bull's actual breeding record, provides the most accurate method of evaluating fertility.

A bibliography of 89 references is appended.

J.G.A.

846. **New Portable Equipment for Measuring Respiration of Sperm Cells.** R. E. COMSTOCK, Univ. Minnesota, St. Paul, Minn. Amer. Soc. Anim. Prod. Proc., 33: 216-220. 1940.

Apparatus is described for field measurement of respiration of semen samples. The principle is the same as for the Haldane gas analysis and the Bancroft apparatus (modified by Dixon) for direct measurement of volume change. In comparison with the Warburg method the correlation coefficient was .88. Corrections were made for temperature and barometric pressure. The equipment is portable and moderate in cost.

G.C.W.

847. **The Site of Elaboration of the Pituitary Gonadotropic Hormone and of Prolactin.** MAURICE H. FRIEDMAN AND S. R. HALL, Bureau of Dairy Industry, U. S. D. A. Endocrinology, 29: 179. 1941.

Beef pituitaries showed a much higher concentration of gonadotropic hormones in the central portion than in the peripheral portion. The reverse was true of prolactin although the difference was not so great. During a time interval when the rabbit pituitary was almost completely exhausted of its gonadotropic hormone content there was no significant change in the prolactin content. It was concluded that prolactin and the gonadotropic hormone were secreted by the rabbit pituitary at independently variable rates and that in both the bovine and the rabbit pituitary, prolactin and the gonadotropic hormone behave as separate and independent physiologic entities.

R.P.R.

848. **Progesterone-like Effect of Ascorbic Acid (Vitamin C) on the Endometrium.** S. LEON ISRAEL AND D. R. MERANZE, Mount Sinai Hospital, Philadelphia. Endocrinology, 29: 210. 1941.

Infantile rabbits and ovariectomized adult mice, rats and rabbits were primed by means of daily injections of estrogen for 7 days. The experi-

mental groups were then injected daily with monoethanolamine salt of ascorbic acid for 5 days. The control animals received either no further injections (estrogen controls) or daily injections of progesterone for 5 days (progesterone controls). The uteri of the 3 groups were then compared as to weight, gross appearance and microscopic characteristics. The progesterone controls developed the heaviest uterine horns, the estrogen controls the lightest and the vitamin treated animals were intermediate. Microscopically the endometrium of the vitamin treated animals approximated the secretory changes of the progesterone group. This response was most marked in infantile rabbits.

R.P.R.

849. **Variations in the Yield of Gonadotropic Material from Green Plants in Relation to the Season of Growth and the pH of the Fresh Juice.** MAURICE H. FRIEDMAN AND JOHN W. MITCHELL. Bureau of Dairy and Plant Industry, U. S. D. A. *Endocrinology*, 29: 172. 1941.

In the experiments conducted there was a seasonal variation in the yields of gonadotropic activity from the juice of immature Sudan grass, oat and corn plants. Inactive juices were obtained from plants in autumn, winter and spring and these were characterized by a high initial pH and a high yield of solids in the washed and dried benzoic acid precipitate. Active juices were obtained from plants in the summer months and these were characterized by a low initial pH and a low yield of solids in the precipitate. Most of the active samples, however, were of relatively low potency, averaging about 1/20 of the potency of the late pregnancy urine.

R.P.R.

850. **The Reproductive Organs in Malnutrition. Effects of Chorionic Gonadotropin upon Atrophic Genitalia of Underfed Male Rats.** MICHAEL G. MULINOS AND LEO POMERANTZ, Dept. Pharmacology, Columbia Univ. *Endocrinology*, 29: 267. 1941.

The effects of acute starvation and of chronic underfeeding on the testes and the accessory genitalia of the male rat were studied. During chronic inanition there was atrophy of the accessory sex organs and of the interstitial cells of Leydig. The loss in weight of the testes and the diminution in the size and spermatogenic activity of the seminiferous tubules were considerable. These regressive changes were corrected by the injection of chorionic gonadotropin. Small doses over a 40-day period being more effective than large doses over a shorter period of time. Likewise, inanition sterility was corrected by the injection of chorionic gonadotropin. Complete starvation for 7 days lowered testicular weight as well as the weight of the accessory genitalia, particularly that of the seminal vesicles.

R.P.R.

851. Use of Stilbestrol in the Suppression of Lactation. SAMUEL D. SOULE AND A. R. BORTNICK, St. Louis, Mo. Jour. Clin. Endocrinol., 1: 409. 1941.

A group of 50 postpartum women were treated with stilbestrol in an attempt to suppress lactation. Five mg. of stilbestrol were administered once, twice or three times. Breast engorgement was relieved or inhibited within 16 hours in 30 of 40 patients in the early puerperium. Treatment after the second week of puerperium, after lactation was well established, did not prove satisfactory. R.P.R.

852. Clinical Effect of Stilbestrol on Postpartum Activity of the Mammary Glands. A. W. DIDDLE AND W. C. KEETTEL, State Univ. of Iowa. Jour. Clin. Endocrinol., 1: 494. 1941.

One hundred postpartum women were treated with stilbestrol for breast engorgement. Forty-six were multiparas and 54 primiparas. Treatment was continued from 1 to 6 days and the dosage varied from 5 to 50 mg. When the administration of stilbestrol was begun within 24 hours postpartum and continued for 5 days, 10 mg. daily, breast engorgement was prevented in most cases. The initiation of lactation was usually delayed but not entirely inhibited. When lactation had been established the amount of engorgement was not noticeably altered by stilbestrol treatment, however, milk secretion was appreciably diminished. R.P.R.

853. Studies on Pituitary Lactogenic Hormone. III. Solubilities of Sheep and Beef Hormones. CHOH HAO LI, WILLIAM R. LYONS AND HERBERT M. EVANS, Univ. California. Jour. Gen. Physiol., 24: 303. 1941.

The solubility of sheep pituitary lactogenic hormone in 0.302 N NaCl at pH 2.02 (solution in HCl) was determined at room temperature. The hormone showed a constant solubility in the presence of a considerable excess of solid phase, indicating that the preparation contained but one component. Beef lactogenic hormone showed a constant solubility in distilled H₂O at 7-8° C. in the presence of excess of the solid phase. The salting-out effect of NaCl in acid solution of both sheep and beef hormones was studied at room temperature. Both preparations behaved as pure substances, but they exhibited differences in solubility, thus indicating a species specificity. R.P.R.

854. Inhibition of Lactation. Percutaneous Use of Testosterone. ABRAHAM J. FLEISCHER AND J. IRVING KUSHNER, Bronx Hospital, New York City. Jour. Clin. Endocrinol., 1: 407. 1941.

An attempt was made to inhibit lactation in 100 postpartum women with

testosterone propionate. Four mg. were administered by inunction daily, beginning on the second postpartum day. (The authors fail to mention the number of daily administrations employed.) The treatment was considered effective in 68 per cent of the cases. It was partially effective in 29 per cent of the cases and was entirely without effect in 3 per cent of the cases. In not one case was lactation entirely arrested, the effect being a diminution in the degree of lactation with resulting diminution of secondary signs and symptoms.

R.P.R.

855. Does Pregnancy Suppress the Lactogenic Hormone of the Pituitary.

C. W. TURNER AND JOSEPH MEITES, Univ. Missouri. *Endocrinology*, 29: 165. 1941.

Experiments were carried out in an attempt to determine whether or not there was an actual suppression of the lactogenic hormone during pregnancy. The lactogen content of the pituitaries of 10 lactating rabbits 20 days postpartum was similar to that of a group of 10 lactating and pregnant rabbits, 20 days postpartum. The average lactogen content per pituitary of rabbits ovariectomized on the 20th day of pregnancy and killed 2 to 5 days later was not significantly different from the amount present in the pituitaries of intact animals on the 20th day of gestation. The lactogenic hormone was detected in the placentas of 3 of the pregnant-lactating group. The authors conclude that pregnancy has no inhibitory effect on the lactogenic hormone of the pituitary.

R.P.R.

856. Influence of Epinephrin and Adrenal Cortical Extract on the Lactogenic Properties of Prolactin.

DAVID R. CLIMENKO AND EVANS W. MCCHESENEY, Res. Labs., Winthrop Chemical Co., Rensselaer, N. Y. *Endocrinology*, 28: 710. 1941.

A study was made of the effect of epinephrin and an adrenal cortical extract (Wilson and Company) on the lactogenic response of the lactogenic hormone. A series of 158 albino rats were rendered pseudo-pregnant by the administration of chorionic gonadotrophin. A slight degree of secretory activity was induced in the mammary glands by the treatment and the administration of 50 I.U. of the lactogenic hormone considerably augmented the effect. A somewhat greater effect was produced by 100 I.U. of lactogen but no significant increment of glandular activity was noted by raising the dose to 150 I.U. The secretory activity obtained by the administration of 50 I.U. of lactogen was somewhat augmented by the simultaneous administration of adrenal cortical extract. A considerably greater effect was obtained if the lactogen treatment was accompanied by an epinephrin-induced state of hyperglycemia. Supplementation by both cortical extract and epinephrin did not seem to produce as great an effect as that produced by supplementation with epinephrin alone. It was suggested that the effect of

the cortical extract might possibly be explained on the basis of its anti-insulin properties. R.P.R.

857. "Mammogen" and the Treatment of Spayed Hypophysectomized Rats with Lipoid Extracts of Cattle Pituitary. ROY O. GREEP AND HOMER E. STAVELY, Div. of Pharmacology and Organic Chemistry, Squibb Inst. for Med. Res., New Brunswick, N. J. *Endocrinology*, 29: 18. 1941.

Female cattle pituitaries desiccated in vacuo, powdered, and injected as a suspension in saline induced mammary growth in spayed hypophysectomized rats. A lipoid fraction, however, produced none of the signs of mammary stimulation. The mammogenic activity of the tissue residue was somewhat reduced by the extraction procedure but this loss of activity was not accounted for in the lipoid fraction. The pituitary tissue, before and after extraction, produced body growth. Increases in body weight and the extent of mammary stimulation were not always correlated. R.P.R.

858. Effect of Stilbestrol on the Mammary Gland. A. A. LEWIS AND C. W. TURNER, Univ. Missouri, Columbia, Mo. *Amer. Soc. Anim. Prod. Proc.*, 33: 63-66. 1940.

Stilbestrol, a synthetic chemical, was reported to have the ability to stimulate the growth of mammary ducts, and some lobule-alveolar tissue, but differs from estrogen in having ability in addition to stimulate milk secretion in virgin or dry animals. It did not consistently stimulate animals already in lactation. G.C.W.

859. The Time of Ovulation and Rate of Spermatozoa Travel in Cattle. J. E. BREWSTER, R. MAY AND C. L. COLE, Michigan State College, East Lansing. *Amer. Soc. Anim. Prod. Proc.*, 33: 304-311. 1940.

The average time of ovulation was 13.57 ± 0.68 hours from the end of estrus in 53 cases of beef and dairy cows as determined by palpation. Heifers ovulated 3.04 hours earlier than cows that had previously calved. Ovulation occurred at any time during the day or night. Some of the cows were slaughtered after artificially inseminating with a bull producing active spermatozoa. Measurements on different parts of the genital organs revealed that the length of the tract was definitely longer for cows than for heifers. The average time required for sperm to reach the upper tube in cows was $5\frac{1}{2}$ hours and in heifers $4\frac{1}{2}$ hours. The rate of travel appeared to be slightly slower in beef than in dairy cows. G.C.W.

860. The Surgical Anatomy of the Teat of the Cow. H. L. FAUST, Dept. Vet. Anat., Iowa State College. *Jour. Amer. Vet. Med. Assoc.*, 98: 143. 1941.

The anatomical structure of the bovine teat is carefully described in a

paper which is well illustrated with pictures and drawings. Schematic drawings of several of the stages in embryonic development of the teat are also shown.

F.E.

MISCELLANEOUS

861. **The Competitive Position of Dairying in Michigan.** ROSS V. BAUMANN AND E. B. HILL, Mich. Agr. Expt. Sta., East Lansing, in cooperation with the U. S. D. A. Bur. of Agr. Econ., Spec Bul., 209. June, 1941.

This study was made in the effort to provide information concerning the outlook for dairying in Michigan. The advantages of dairying, compared with other lines of production, under conditions likely to develop in the future are considered. Estimates of future production are made over a ten-year period under different price conditions and also assuming there is no change in price relationships. Two areas were studied in detail and the results extended to surrounding regions by comparisons of farming systems and other conditions.

A continuation of the present trend toward more milk production is indicated by a budget analysis of individual farms. Additional feed is expected to become available as a result of greater use of alfalfa and hybrid corn.

P.H.T.

862. **How to Figure Refrigeration Insulation.** Refrigeration Engineering Application Data 27. LEE C. LESLIE, Philadelphia, Pa. Refrig. Engin., 42, No. 2: 136. 1941.

The author gives details in arriving at economic thicknesses of insulation citing practical problems and accompanying his explanations with tables and graphs. Attention is given to moisture resistance and protection and proper methods of installation of slab and bulk insulation materials. Numerous tables of valuable material are also included.

L.M.D.

863. **Locker Storage and Related Freezing Facilities for Community Storage Plants.** W. R. WOOLRICH, Univ. Texas, Austin, Texas. Ice and Refrig., 100, No. 3: 199. 1941.

This paper traces the historical development of locker storage plants and also announces the development by the University of Texas Bureau of Engineering Research of a new freezer for quick freezing of vegetables and fruits at a cost of $2\frac{1}{2}$ cents (including preparation of the product) per pound. A description of the types of locker plants is included. Some plants offer a multiplicity of services, e.g., (1) Slaughtering of livestock, (2) chilling of meats, (3) curing of meats, (4) slicing and wrapping of meats, (5) sausage

making and lard rendering, (6) smoking of meats, (7) fruit, vegetables, and fish processing, (8) sharp freezing, (9) quick freezing, (10) storage and (11) selling and buying of products.

A discussion of plant design is included. The chill room should be at least 12 ft. high and be insulated for a temperature of 30° F. This calls for 5 or 6 inches of cork or its equivalent. The area of the chill room should be about $\frac{1}{2}$ sq. ft. per anticipated locker capacity. The outside wall and ceiling of the sharp freezer room should have 8 inches of cork or its equivalent while the inner walls between locker, chill room and processing rooms should have at least 6 inches since this room is designed for a minus 15° F. temperature. It should have a minimum of 60 sq. ft. of floor space for a 300 locker plant plus $\frac{1}{10}$ sq. ft. for each additional locker. The locker room temperature should be near 0° F. in order to minimize odors. The aisles should be 30 inches wide. Ordinarily the dimensions should be 2.5 to 3 sq. ft. per locker installed. Load concentrations on the floor may reach 400 to 500 lbs. per sq. ft. Odorless asphalt should be used in the construction of locker rooms.

Refrigeration requirements average 1 ton per 50 lockers in the south or per 60 lockers in the north.

L.C.T.

864. The Conservation of Water in Refrigeration. GEORGE E. TOLES.
Ice and Refrig., 100, No. 2: 109. 1941.

This article resolves itself into a justification for the use of evaporative condensers in preference to all other kinds as a means for cutting down on condensing water costs. Figures are given for relative savings on a 50 ton plant if different condensers are used. It is pointed out that with a cooling tower 4 gallons of water per minute per ton capacity must be circulated, while an evaporative condenser will use but $1\frac{3}{4}$ gallons of water per hour plus a 25 per cent entrainment. In the case of a city water condenser, 1 gallon per minute per ton is the usual usage. Even though air circulating fans are required with the latter system, the final operating cost will probably be 25 per cent that of the city water system and 35 per cent of that of the cooling tower system. Proper engineering can make the evaporative condenser fully automatic in operation.

Where the evaporative condenser is not used, savings in water may be effected by using the condenser water for other purposes.

L.C.T.

865. Census Bureau Reports on Refrigerating Business. ANONYMOUS.
Ice and Refrig., 100, No. 2: 97. 1941.

The article in question consists entirely of a mass of data giving production figures and values of mechanical units and value of ice manufactured. Machines are classified according to power usage. Employment figures for the refrigerated machinery industry are given.

L.C.T.

866. Construction of Flooded Evaporators for Stable Operation. F. J. BOBBY, The Union Ice Co., Watsonville, Calif. *Ice and Refrig.*, 100, No. 1:32. 1941.

Flooded evaporators should meet certain requirements, *e.g.*:

1. The unit must be arranged in such a way as to operate with a low liquid head to obtain the lowest temperatures of the refrigerant for any given evaporating pressure.
2. It must produce proper circulation of the liquid refrigerant in order to wash the gas film from the transfer surfaces.
3. It must permit proper refrigerant velocities in the pipe coils in order to avoid excessive friction and turbulence.
4. It must be arranged in such a way as to provide the proper liquid head in order to give this desired circulation.
5. It must be provided with adequate return pipes for the circulating liquid (drop legs) in order to return the circulating liquid to the evaporator and avoid piling up under maximum load conditions.
6. It must provide either fixed or variable liquid level control in order to fix the operating head under any given load and to protect against flooding.

The author points out that 50 per cent vapor to total gas and liquid by volume is about optimum. A velocity of 300 ft. per minute in the piping is desirable. In order to maintain the proper velocity a certain amount of head must be maintained. This can be determined by use of certain formulae which the author includes. Operating diagrams also are given. L.C.T.

867. Piping Design Problems in Frozen Foods Locker Plants. JOHN H. CARTER, Kupferle-Hicks Heating Co., 3974 Delmar Blvd., St. Louis, Mo. *Ice and Refrig.*, 99, No. 4: 271. 1940.

This article includes a floor plan for a 415 locker plant. Temperatures and times which are most desirable are listed as follows: Chilling, 33° to 35° F. for about 24 hours; aging, 33° to 35° F. for 3 days for hogs, ordinary beef a week to 10 days, and well fattened corn-fed steer beef as much as three or four weeks; freezing, -15° F. as low as 12 minutes for peas, and as much as 12 hours for a large roast; storage, 0 to 12° F. with 0° to 5° most desirable, and average time about 3 months. The usual loading allowance is 3 pounds of product per locker per day, somewhat less in Summer, and somewhat more in Fall. For a 415 locker plant as illustrated, the estimated load for the locker room alone is 10,878 btu. per hour maximum. The locker room would require about 800 sq. ft. of 1½ inch F.W.B.S. piping, or about 350 sq. ft. of coil surface. If the coil surface is free from frost and a transfer factor of 2.5 btu./hr./sq. ft./deg. temp. difference is assumed, a temperature difference of 12.5° F. must be maintained. As frost accumulates this difference must be increased or the coil area must be increased beyond 800 sq. ft., *e.g.*, to 1200 sq. ft.

For the freezer room with a maximum requirement of 16,625 btu./hr., about 200 sq. ft. of piping will be required if forced circulation at the rate of 500 ft./min. is used, and the transfer factor is 7.0. About 8 to 12 air changes per minute are recommended. The capacity of the blower can then be readily estimated. Coils should be defrosted frequently. About 600 ft. of 1½" F.W.B.S. pipe coil are advised for the aging room. This is based on a 4221 btu./hr. load. The chill room requires about 435 ft. of 1½" F.W.B.S. pipe coil, based on a 3011 btu./hr. load. The general storage room with a 3515 btu./hr. load will take about 325 ft. of 1½" F.W.B.S. pipe coil. With a load of 2902 btu./hr. the curing room will require about 205 ft. of 1½" F.W.B.S. pipe coil.

All figures in this article were based on the use of ammonia as the refrigerant, and would require revision if others were used. L.C.T.

868. Priorities and Your Business. ANONYMOUS. Ice Cream Field, 38, No. 2: 39. 1941.

Mention is made of the fact that many products, including aluminum, nickel-bearing steel, milk steel, rubber, synthetic rubber, and electric motors are now on the priority list of the Office of Production Management in Washington. Other additions are being made to this list and the Dairy Industries, themselves essential industries, are finding it more difficult to operate.

Those responsible for the nation's planning realize the importance of dairy products to the health and well being of her population and are doing what they can to enable the Dairy Industries to continue to operate although very definite restrictions and changes have been made in supplies and equipment, and others will of necessity follow. So far many of these restrictions are on a voluntary basis and it is stated that if handled properly this can continue.

One of the demands of the O.P.M. is that all industries attempt to work out suitable substitutes to relieve shortages of critical materials. For the industrial equipment and supplies field this program, it is stated, has been assumed by a special sub-committee of the Dairy Industries Supply Associations Technical Committee. W.C.C.

869. Raising the 4-H Dairy Calf. Raising the 4-H Dairy Heifer. Managing the 4-H Dairy Cow. E. J. PERRY AND J. R. PORTER. New Jersey Agr. Ext. Serv. Bul. 225, 226, and 227, respectively. 11 pages, 14 pages, and 18 pages, respectively. 1941.

This series of attractively illustrated bulletins written especially for 4-H club members gives specific information on the topics indicated. J.G.A.

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ABSTRACTS OF LITERATURE

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A. J. POWERS, Brooklyn,
New York, I. A. M. D.

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JOURNALS

American Butter Review	Journal of Industrial and Engineering Chemistry
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SPECIAL PUBLICATIONS

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New York Association of Dairy and Milk Inspectors	United States Department of Agriculture

ABSTRACTS OF LITERATURE

ADVANCE ABSTRACTS OF REPORTS ACCEPTED FOR PUBLICATION IN THE JOURNAL OF DAIRY SCIENCE

870. **Distribution of Diacetyl and Acetylmethylcarbinol between Fat and Water, with Special Reference to Butter.** W. H. HOECKER AND B. W. HAMMER, Iowa State College.

In unsalted and salted butter, both the serum and the fat contained diacetyl and also acetylmethylcarbinol. The serum contained higher concentrations of the compounds than the fat, the differences being greater with acetylmethylcarbinol than with diacetyl. In each type of butter, a larger percentage of the total diacetyl than of the total acetylmethylcarbinol was contained in the fat. Butter into which a solution of diacetyl or a distillate of butter culture had been worked showed the same general distribution of diacetyl as butter made from cream containing butter culture.

In general, the data obtained on mixtures of Wesson oil and water or brine and mixtures of butterfat and water or brine agree with the results obtained on butter. In such mixtures, and also in butter although the results were not as definite as with the mixtures, the addition of sodium chloride increased the percentage of diacetyl or acetylmethylcarbinol that was in the fat. The concentration of diacetyl in the mixtures or in butter apparently did not affect the percentage contained in the fat, but as the concentration of acetylmethylcarbinol increased the percentage contained in the fat decreased.

871. **Identification of the White Particles on Ripened Cheddar Cheese.** F. L. DORN AND A. C. DAHLBERG, New York Agr. Expt. Sta., Geneva, N. Y.

A study was made of the white granular particles which commonly appear on the surface and throughout the body of cheddar cheese after 5-6 months ripening. The insolubility of the material in cold water, its low ash and calcium content, and a negative test for the lactate radical eliminated the possibility that the material was calcium lactate. The large amount of phosphorus present in the ash in relation to the calcium suggested a calcium phosphate salt as an impurity or a minor constituent of the white material.

The major constituent of the white particles was tyrosine, the identification of which was made on the basis of its characteristic color reactions, crystal formation, and by the melting point of the dibenzoyl derivative.

872. **The Introduction of Cattle into Colonial North America.** G. A. BOWLING, West Virginia University, Morgantown.

The initial period of importations of cattle into the North American Colonies extended from 1493 to about 1640.

During the period of discovery and colonization there were four possible paths of introduction of cattle into what is now the United States of America. (1) From the West Indies to any portion of the Atlantic and Gulf of Mexico coast line. (2) From Mexico into southwestern areas and California. (3) From the French colonies of the St. Lawrence Valley into the area of the "Old Northwest." (4) Directly from the colonizing European Nations to the American Colonies.

At an early date Spanish cattle were introduced in comparatively large numbers into the entire southern part of our country and they formed the foundation for the cattle breeding industry of that section. When the English, Dutch, Swedish and French colonies were founded in the east and the northeastern part of the continent, most of the cattle were imported directly from the colonizing European countries. After these colonies became established they, in turn, supplied cattle for the later colonies. After 1640 practically all of the American cattle trade, other than local transactions, was on an intercolonial basis, and the colonies as a group were self-sufficient in respect to cattle needs.

Since the initial introduction of cattle ended before the livestock improvement movement got under way in England and the other European countries the North American cattle population, numbering several millions at the end of the American Revolution, was of nondescript breeding. Real improvement of the cattle population of the United States did not begin until the second importation period which was not initiated until after the Revolutionary War.

873. Accuracy of Live Weights of Dairy Cows on Pasture. R. E. HODGSON AND J. C. KNOTT, Bur. Dairy Indus., Washington, D. C.

The experimental error, the standard deviation of daily trends and the standard error of three-day initial and final live weights of cows on pasture have been determined by analyses of variance. The experimental error of 46 weight groups was 14.0 pounds with a range of from 7.0 to 28.3 pounds. The standard deviation of day to day trends of the groups of cows averaged 7.7 pounds with a range from 0.5 to 20.8 pounds. The standard error of the live weights of cows weighing about 1200 pounds averaged only 2.2 pounds. The experimental error, standard deviation of daily weight changes and standard error of the mean initial and final weights were of about the same order.

874. The Anatomy and Physiology of the Teat Sphincter. DWIGHT ESPE AND C. Y. CANNON, Iowa Agr. Expt. Sta., Ames, Iowa.

An abstract of this paper appears on page 500 of the June, 1941, issue of the JOURNAL OF DAIRY SCIENCE.

BOOK REVIEW

875. **The Market Milk Industry.** CHESTER LINWOOD ROADHOUSE AND JAMES LLOYD HENDERSON, Univ. California. McGraw-Hill, New York, 624 pages.

This book of 624 pages published by McGraw-Hill Book Company of New York is a comprehensive treatise of the entire market milk industry. It is a book written in language that can be understood by the entire market milk industry. It is not too technical, yet it contains sufficient technical material to be of aid to the laboratory man who is attempting to get to the bottom of some plant problem.

The book will appeal to milk plant operators from the manager to the man on the milk wagon. It will appeal to the student in dairy departments, to milk inspectors and members of various boards of health.

There are twenty-five chapters in the book and some of the chapters include such subjects as history, composition and properties of milk, micro-organisms, enzymes, etc. An entire chapter is devoted to milk and public health, another to safeguarding milk supply and still another to sanitary production of market milk.

Construction and arrangement of dairy farm buildings as well as city milk plants are dealt with in separate chapters. Country receiving stations, transportation of milk, flavor of milk, milk plant operation, washing and sterilizing, and pasteurizing are covered in individual chapters.

Mechanical refrigeration is discussed briefly. Such subjects as creaming of milk, table cream and whipping cream and special milk products are covered in some detail.

Other chapters discuss distribution of milk (29 pages), cost of milk production, price and price plans, dairy inspection and control, milk in nutrition and the milk plant laboratory and its operation. The appendix contains dairy arithmetic problems, depreciation rates and other useful information.

The book is ably written and well illustrated with photographs, charts, tables and drawings. In all, there are 167 illustrations and 126 tables.

C.D.D.

BACTERIOLOGY

876. **Control of Spreaders.** M. O. ROBINSON, Alabama Polytechnic Inst., Auburn, Ala. Milk Dealer, 30, No. 10: 32-33. July, 1941.

The author found that pouring media too deep in the plates caused trouble with spreaders.

C.J.B.

877. **Methods for the Bacteriological Examination of Milk Bottle Caps, Hoods and Closures.** J. R. SANBORN AND ROBERT S. BREED, New

York State Exp. Sta., Geneva, N. Y. Jour. Milk Technol., 4, No. 2: 63. 1941.

Methods for determining surface contamination of milk bottle caps or closure surfaces are given. The results of studies made show that the bacterial contamination of hood, and closure surfaces is normally very slight. Rinsing and contact culture methods used were found to be generally comparable. Results of over 100 analyses reveal a total absence of coliform organisms.

In view of many different sizes in closures and the low surface counts secured it is difficult to set a bacterial standard. It is suggested that the count be expressed in terms of colonies per unit of surface area. A figure stated was that closure surfaces coming in contact with the product should not have a count in excess of 2 colonies per square centimeter; or a count if not to exceed 10 or 25 colonies per cap or closure would seem reasonable. It was suggested, however, that at first a more lenient standard might be fixed until the problem had been given more study. L.H.B.

878. Effect of Pasteurization on Esch. Coli Organisms. C. PALEY AND M. L. ISAACS, Columbia Univ., New York, N. Y. Canad. Dairy and Ice Cream Jour., 20, No. 7: 19. 1941.

This is a reprint of an article which appeared in the JOURNAL OF DAIRY SCIENCE, 24: 421, 1941. O.F.G.

879. The Sporadic Appearance of Pin-Point Colonies in Raw Milk Counts. M. O. ROBINSON, Alabama Polytechnic Inst., Auburn, Ala. Milk Dealer, 30, No. 9: 32, 44. June, 1941.

An account is given of a sporadic appearance of pinpoint colonies in raw milk counts due to placing a few pasteurized-milk cans through the washer while waiting for the producer cans. C.J.B.

BREEDING

880. Improving Dairy Herds to Lower Milk Production Cost. O. E. REED, U. S. D. A., Bureau of Dairy Industry, Washington, D. C. Canad. Dairy and Ice Cream Jour., 20, No. 5: 26. 1941.

The problem of price reduction to the consumer is the joint concern of both the producer and the distributor. The job of the dairy industry is to help offset the low family incomes by adopting every efficient practice known. The breeding of better dairy cattle is one of the efficient practices. One-third of the heifers saved each year for herd replacements turn out to be poor producers. The real objective of the proved-sire system is not merely to raise the level of herd production but rather to "purify" the germ plasm to such an extent that fewer and fewer offspring will have low-

production inheritance. Production records are the heart of an efficient production system. Rapid developments in artificial insemination have opened up new opportunities in developing better herds. O.F.G.

881. (Hochschule für Bodenkultur, Vienna.) Rassenkundliche Untersuchungen an Schädelresten des altägyptischen Hausrindes. PIA D. J. JULIUS. Ztschr. f. Tierzücht. u. Züchtungsbiol., 48, No. 1: 17-55. (14 figures and 9 tables of skull and horn measurements.) November, 1940.

The skulls investigated are from 3000 years, from 2500 years, and from 1000 years or less B.C. The skulls show prevailingly primigenius characteristics, the important deviations being tentatively imputed to the introduction of Mongolian blood. Among living races of cattle, the Hungarian steppe race most closely resembles these Egyptian cattle. J.L.L.

BUTTER

882. L'abaissement du taux des acides volatils ne rend pas le beurre "anormal." (Reduced Volatile Acid Values not Yielded by "Abnormal" Butter.) L. HIRON. Le Lait, 20, Nos. 198, 199, 200: 497-510. 1940.

Maximum and minimum values of 33 and 26 were fixed in 1888 as being the limits for pure butter when it was subjected to the Reichert-Meissl determination. Butter having an R-M value below 26 was considered adulterated. These values were later questioned and several studies are reviewed indicating that butters of known purity sometimes yielded values outside the above limits and also showed a marked seasonal variation. Comparisons are presented between R-M values and indices of refraction. A negative correlation exists between these values but the relationship is not at all strict.

On a large number of samples analyzed at two week intervals from 1927 to 1938 the maximum R-M values were between 31.5-32.5 and the minimum values between 25.5-27.5, the former occurring during early spring and the latter about mid-autumn. The probable causes of some of these variations are discussed. O.R.I.

883. Mycostatic Salts Prevent Mold Growth on Stored Foods. W. L. MALLMAN, Mich. Agr. Expt. Sta., East Lansing, Mich. Food Indus., 13, No. 7: 41-42, 54. 1941.

How to store foods at refrigerated temperatures for long periods without loss in quality or market value is a great problem to many food producers and distributors.

An essential for mold growth is the presence of moisture to germinate the spores and to start the mycelial growth. Such a condition exists when the humidity of storage room is sufficient to form a film of moisture on the surface of foods and their containers. When the humidity is controlled to prevent the formation of moisture, the foods lose moisture to the air. Shrinkage and loss of food quality result.

When moldy foods were examined it was found that penicillia type mold (green mold) dominated. It was also found that enough mold to cause trouble was brought into the storage room on the food themselves.

It was found that mold spoilage could be controlled by concurrent disinfection. This may be accomplished in several ways. Carbon dioxide and small amounts of ozone are two ways of doing this at reasonable price. Introduction of a chemical agent, sodium pentachlorophenate, into the packing materials is a method which has proved effective for foods stored for three to six months.

J.C.M.

884. How to Improve the Keeping Quality of Butter. O. F. HUNZIKER, La Grange, Ill. *Canad. Dairy and Ice Cream Jour.*, 20, No. 7: 23. 1941.

Good butter is made from high quality cream. The cardinal essentials for high quality in cream are sanitation, low temperatures and freshness, all of which start on the farm and must be practiced until the final product reaches the consumer. Efficient pasteurization accompanied by a sanitary status as to equipment, plant and all operations, that will protect the cream between pasteurizer and churn, and the butter between churn and consumer package, from every potential channel of recontamination are essential. Chemical deterioration which results in fishy, tallowy, metallic and storage flavors is generally hastened and intensified by the presence of salt, high acidity and metallic compounds. Unsalted butter is more stable chemically but is highly perishable bacteriologically.

O.F.G.

885. Leakiness in Butter. S. T. COULTER, Univ. Minnesota, St. Paul, Minn. *Canad. Dairy and Ice Cream Jour.*, 20, No. 7: 66. 1941.

The appearance of a plug of butter drawn with a trier is not a reliable index to whether or not the butter will lose moisture during and after printing. The Coulter and Combs method, described in this article, has been found most useful. Among the factors which influence the water retaining properties of butter are: (1) Composition of the butterfat—winter butter retains more water than summer butter, and (2) Methods of manufacture. The factors involved under methods of manufacture are (a) thoroughness of cream cooling, (b) temperature of the butter during working, (c) stage of working at which water for standardization is added, (d) salting at the

granular stage or after the butter has been worked into a roll, (e) amount of working, and (f) composition of the butter. O.F.G.

886. **The Value of Mold and Yeast Control Work.** E. G. Hood, Dept. Agr., Ottawa, Canad. Canad. Dairy and Ice Cream Jour., 20, No. 4: 29. 1941.

The main sources of molds and yeasts in butter are: (1) Inefficient pasteurization and raw cream recontamination. (2) Dead ends in pipes. (3) Improperly cleaned and sterilized vat, pumps and pipes. (4) Improperly cleaned and sterilized churns. (5) Impure water, unclean holding tanks and ice. (6) Improperly treated parchment linings. (7) Butter boxes—unseasoned lumber and damp storage.

The Storch test is not sufficient evidence for satisfactory pasteurization of cream and needs to be supplemented by yeast and mold counts. Pasteurized butter should contain less than a total of 50 molds and yeasts and 25,000 bacteria per gram of butter where starters are not used. The mold and yeast count has been found to be an index of keeping quality of the butter. O.F.G.

887. **A Summary of Recent New Zealand Research Work on Buttermaking.** ANONYMOUS. Canad. Dairy and Ice Cream Jour., 20, No. 4: 64. 1941.

Fat losses in churning—Results from estimating fat losses by weighing and analyzing the cream, buttermilk and wash water agreed well with the methods used by Udy and by Bird and Derby.

Fat oxidation—Butters made from ripened cream oxidized more rapidly than corresponding butters made from cream acidified to the same extent with pure lactic acid. Salted butters oxidized more rapidly than corresponding unsalted butters. Low pasteurization temperatures favored oxidation. The presence of an oxidizing enzyme was indicated.

Butter starters—Starters are not used in New Zealand for “ripening” cream but a small amount is used by some factories to give a better flavor to butter made from sweet cream. *Str. cremoris* is used to give a good flavor.

Hardness of butter—The Blair instrument has given useful indications of butter hardness although it is not without criticism.

Rate of cooling cream—The more rapidly cream is cooled after pasteurization the harder is the butter. Seasonal and even daily variations were greater than variations produced by different methods of treatment.

Antioxidants—Vitamins C and E appeared to have antioxygenic effects. Soluble phosphates and citrates of the milk plasma gave striking positive results. O.F.G.

888. **Research and the Creamery Industry.** H. MACY, Univ. Minnesota, St. Paul, Minn. *Canad. Dairy and Ice Cream Jour.*, 20, No. 5: 66. 1941.

This article is an account of the scientific practices which have been used to improve the quality of creamery butter. The use of the propionates in retarding or preventing the growth of surface molds on printed butter is an example of these practices. The use of carbon dioxide has not proved successful in storing butter. The putrid or cheesy surface taint has frequently been traced to water supplies. Some waters have required so much chlorine to destroy the causative organisms that a chemical flavor has been imparted to the butter and made it unsaleable. Experiments with cereal antioxidants at Minnesota have not proved particularly successful. The mold mycelia count is practically an infallible test to determine whether or not butter has been made from a cream of poor quality. A test has been developed for use in the creamery receiving room to detect molds. O.F.G.

889. **Water, Churn Sanitation and Butter Flavor.** M. C. JAMIESON, Univ. Manitoba, Winnipeg, Man. *Canad. Dairy and Ice Cream Jour.*, 20, No. 6: 20. 1941.

Seventy-one per cent of bacteria whose colonies fluoresced under ultra-violet light produced butter with flavor scores under 37. Over 70 per cent of oxidase-positive bacteria were responsible for flavor scores of 37 or lower. Out of 65 cultures classed as probable surface taint producing type 56.9 per cent were definitely so; 43.1 per cent caused a cheesy to rancid flavor, while 15.4 per cent produced an unclean taste. Poor sanitation in a churn can off-set the best water supply and lower flavor scores in butter. Skill in manufacture can overcome some defects in either water or sanitation. The majority of fluorescent and oxidase-positive bacteria are more deleterious to butter flavor than types otherwise distinguishable from these in colony growth. O.F.G.

890. **The Significance of Yeast and Mold Counts.** A. G. LEGGATT, Ontario Agr. College, Guelph, Ont. *Canad. Dairy and Ice Cream Jour.*, 20, No. 6: 23. 1941.

The yeast and mold count of butter is a measure of the creamery sanitation. The test is dependent upon taking the samples as follows: (1) Take sample from butter that has been worked, (2) Make up sample with as many small pieces and from as many locations in the mass of butter as is possible, (3) Take the sample quickly, (4) Replace the cover of the sample jar immediately, (5) Do not allow the hand or any part of the clothing to pass over the mouth of the jar while open, and (6) Always hold the cover of the jar topside up. Where a creamery runs high with its yeast and mold counts a

line check should be made. A line check consists of a series of samples taken from the same batch of cream so as to follow it through the various processes until it is manufactured into butter. The churn is the chief source of contamination.

O.F.G.

891. **Observations on the Use of the "Vacreator."** H. W. NICHOL, Saskatchewan Co-operative Creameries, Ltd., Regina, Sask. Canad. Dairy and Ice Cream Jour., 20, No. 6: 64. 1941.

Number 1 butter can be made from cream with feed and volatile weed flavors by pasteurizing the cream in the "vacreator." Cream with yeasty, cheesy, metallic or stale flavors cannot be made into No. 1 butter by "vacreating" although the flavor of this butter will be greatly improved. Warning is issued, therefore, against becoming lenient on the grading of cream for buttermarking purposes. The "vacreator" has done an excellent job in preserving the natural flavors of butter and has greatly improved the keeping quality. Surface flavor on butter has been almost entirely eliminated. The body and texture of the butter are improved. The use of the "vacreator" resulted in a reasonable saving in water and a definite saving in power and fuel.

O.F.G.

CHEESE

892. **Controlling Mold Growth on Cheddar Cheese.** C. K. JOHNS, Central Experimental Farm, Ottawa, Can. Canad. Dairy and Ice Cream Jour., 20, No. 4: 36. 1941.

Cheddar cheese swabbed with 4 per cent and 8 per cent solutions of calcium propionate showed slight molding but their condition was vastly superior to that of the control cheese. The cheese treated with 12 per cent solution remained free from mold growth. Calcium propionate does not kill molds—it merely checks their growth. Dipping of the cheese in solution probably gives more efficient application but has certain disadvantages which do not make for simplicity.

O.F.G.

893. **Curing and Merchandising Cheese in Sealed Packs.** H. L. WILSON, Bureau of Dairy Industry, U. S. D. A., Washington, D. C. Canad. Dairy and Ice Cream Jour., 20, No. 7: 28. 1941.

The greatest difficulty in curing cheese in sealed containers is the uncertainty of the quality of the curd when packed. Results show that when the methylene blue reduction time of the milk is less than 3 hours a high percentage of undergrade cheese is made. Only a small percentage of milk received at cheese factories during summer months meets this requirement. Pasteurization of milk results in a better and more uniform cheese, especially if the milk does not meet the minimum reduction time of 3 hours. The con-

trol of acidity is the greatest quality controlling factor. Directions are given for making a cheese having a smooth, fine and waxy body, a texture free from mechanical and other openings, and a pleasant characteristic nutty flavor.

O.F.G.

894. **Salt for Cheesemaking.** L. B. BRYANT, Ontario Agr. College, Guelph, Ont. *Canad. Dairy and Ice Cream Jour.*, 20, No. 4: 21. 1941.

Cheese salt should be clean, of a high grade of purity, coarse-grained, flaky and free from lumps because it dissolves more easily. Thirty-four samples of cheese salt were subjected to physical, chemical, bacteriological and cheese manufacturing tests. Twenty-two of these salts were not sufficiently coarse for cheesemaking. Bacteria counts ranged from 0 to 42,000 per gram of salt, molds ranged from 2 to 40 per gram, but no yeasts were found. Many of the samples contained considerable amounts of foreign material. In manufacturing tests, the rate of solubility and draining off whey in the salting operation were both more rapid in the case of fine salts. Cheese to which the coarse salt was added showed the greatest loss of salt and the smallest loss of protein and fat in the wheys draining off from the salting vats and press, but these losses were not large enough to cause a significant difference in the composition of the final cheese. The scores on cheese made from fine or coarse salt were not appreciably different.

O.F.G.

895. **Meeting the Demand for Imported Type Cheese.** O. R. IRVINE, Ontario Agricultural College, Guelph, Ont. *Canad. Dairy and Ice Cream Jour.*, 20, No. 4: 80. 1941.

Dislocation of the international cheese trade has affected Canadian imports of the following types: Roquefort, Danish blue, Gorgonzola, Gruyere, Emmenthaler, Gouda, Edam, Parmesan, Camembert and Brie. This situation has created a market for Canadian cheesemakers. The chance to introduce Canadian made replicas of these imported cheese is dependent upon technical difficulties which must be overcome. The possibilities of making Cheshire, Edam and Gouda, and the blue types of cheese in Canada seem promising.

O.F.G.

896. **A Review of the Ontario Cheese Industry.** H. B. SANDWICH, Chief Cheese Instructor for Eastern Ontario. *Canad. Dairy and Ice Cream Jour.*, 20, No. 5: 23. 1941.

Factors which have tended to improve the cheese industry in Canada during the past season are: (1) The Cheese and Cheese Factory Improvement Act and subsidies, (2) Greater co-operation between producer and cheesemaker, (3) The change in instructional work, (4) Favorable weather

conditions, and (5) General improvement in cheese factories under license system. Under the Cheese Act the standard of the cheesemaker has been raised. The total number of undergrades of cheese, due to mechanical defects, has been reduced. Flavor defects have appeared less frequently.

O.F.G.

897. **Factors Affecting the Quality of Cheese.** S. L. TUCKEY, Univ. Illinois, Urbana, Ill. *Canad. Dairy and Ice Cream Jour.*, 20, No. 6: 30. 1941.

Flavor in cheddar cheese is the result of (1) bacterial development, (2) acidity development, (3) moisture content, and (4) salt content. Under conditions of too much acid, bitter flavor defects predominate but this flavor also may occur under other conditions. In studies at the University of Illinois cheese made with a salt content of 1.7 per cent was never criticized as being bitter. It was also noted that this cheese was slower in breakdown of body than cheese with a salt content of 1.2 per cent. Cheese that is to be aged for 6 to 12 months probably should have more salt than cheese which is to be sold on the market fresh with a mild flavor. Butterfat contributes to the characteristic flavor of cheese but the use of a portion of homogenized raw or a mixture of enzymes to accelerate the splitting of fatty acids has resulted in the development in the cheese in a short time of too much rancidity.

O.F.G.

898. **Bacterial Cultures for Cheese Made from Pasteurized Milk.** W. C. HARRIS, B. W. HAMMER AND C. E. LANE, Iowa State College, Ames, Iowa. *Canad. Dairy and Ice Cream Jour.*, 20, No. 6: 70. 1941.

Thirty-four cultures of micrococci isolated from cheddar cheese where studied. They were grouped into 23 species 12 of which could be identified. Cheese was made from milk cultured with these 23 species. On examination of the cheese it was found that they could be divided into 3 groups according to flavor: (1) those having an undesirable effect, (2) those having no definite effect, and (3) those having a desirable effect. Thirteen of the cultures appeared to improve the flavor of the cheese. The results suggested that certain strains of micrococci may be useful in making cheese from pasteurized milk but selection of the culture should be made on the basis of strain rather than species. The results also indicated that certain strains of propionic acid bacteria may be useful in the manufacture of cheddar cheese from pasteurized milk.

O.F.G.

CHEMISTRY

899. **Methods of Determining Vitamin B in Foods.** EARL D. STEWART, Consumer's Yeast, Oakland, Calif. *Food Indus.*, 13, No. 7: 56-61. 1941.

This article deals with the determination of vitamin B₁ in bread and

bakery goods but the factors to be kept in mind will be much the same for any food product.

The author goes into very lengthy discussions on the different ways that have been derived to test for vitamin B₁.

None of these methods are very desirable as to general applicability or reliability as yet. With increased activity in this field better methods are certain to come. J.C.M.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

900. **The Development of New Methods in Dairy Science.** P. F. SHARP, Cornell Univ., Ithaca, N. Y. *Canad. Dairy and Ice Cream Jour.*, 20, No. 4: 24. 1941.

The author discusses the new developments and newer knowledge of the processing, distribution and nutritive value of milk. Examples of recent research findings are illustrated by discussing some of the products and by-products of milk such as frozen cream, dried whey, casein, etc. He feels that opportunities for research in the field of dairy products are almost infinite. O.F.G.

901. **The Whey Problem.** J. G. DAVIS, Natl. Inst. for Res. in Dairying, Shinfield, nr. Reading, Eng. *Canad. Dairy and Ice Cream Jour.*, 20, No. 6: 72. 1941.

The whey problem arose with the advent of factory cheese making. Whey has been used in its unchanged form for hog feeding, for whey silage and for conversion to whey beverages. It has been dried in the form of whey paste, whey powder and for extraction of lactose. It has been boiled to form albumen cheese. It has been microbiologically utilized by fermentation to lactic acid, fermentation to alcohol and as a medium for yeast for cattle food. It is particularly rich in calcium and vitamin B₂ (vitamin G or riboflavin) as well as lactose and is, therefore, a valuable source of human nutrients. There is need for further developments of methods of utilizing whey. O.F.G.

902. **L'utilisation du sérum. (The Utilization of Whey.)** G. GENIN. *Le Lait*, 20, Nos. 198, 199, 200: 510-517. 1940.

This is a description of the process developed by Sheffield Farms for the extraction and purification of lactose and precipitation of albumin.

O.R.I.

FOOD VALUE OF DAIRY PRODUCTS

903. **Some Trends in Consumption of Dairy Products.** E. E. VIAL, Bur. Agr. Economics, U.S.D.A. *Milk Dealer*, 30, No. 9: 118-123. June, 1941.

A discussion is included of the trend in the consumption of dairy prod-

ucts. Data are presented showing the *per capita* consumption of dairy products; changes in consumption of milk in selected Northeastern markets, 1936-40; relation between income and consumer purchases of dairy products, and the relation between income and consumer purchases, in pounds, of fruits, vegetables, meats, dairy products, and eggs.

The effect of the defense program on the consumption of dairy products is also discussed. The author concludes that it seems quite probable that during the next year or two our total consumption of dairy products will increase.

C.J.B.

904. **Better Meals for Tomorrow.** LEWIS W. WATERS, General Foods Corp., New York City. Jour. Milk Technol., 4, No. 2: 68. 1941.

A suggested program for the food industry is given which should assure better meals for tomorrow. The program is as follows: 1. Use the best materials. 2. Manufacture efficiently. 3. Process carefully. 4. Package properly. 5. Distribute widely. 6. Advertise honestly. 7. Price wisely. 8. Supply directions. 9. Strive for convenience. 10. Maintain constant research.

L.H.B.

905. **Le lait frais pasteurisé irradié a l'abri de l'air et la vitamine D indications médicales—posologie.** (Fresh Milk Pasteurized and Irradiated Protected from Air; the Medical Requirements and Dosage of Vitamin D.) JEAN VIEILLY. Le Lait, 20, Nos. 198, 199, 200: 517-527. 1940.

Literature is reviewed and many opinions cited (although no bibliography is included) on methods of providing increased supplies of vitamin D particularly by the irradiation process. Some of the symptoms of hypo- and hyper-vitaminosis D and hypercalcemia are discussed especially in relation to therapy by irradiated ergosterol.

It is pointed out that the irradiation process as applied to milk takes place rapidly with no possibility for toxic products to be developed. The process should be limited to milk. Fresh, pasteurized, irradiated milk is an excellent source of this vitamin for expectant mothers and infants. O.R.I.

906. **How to Add Vitamin D Concentrate and Control Vitamin D in Milk.** C. I. Post, Natl. Oil Products Co., Harrison, N. J. Food Indus., 13, No. 6: 41-43. 1941.

This subject falls into three phases: (1) Control of the vitamin D potency of the concentrate; (2) Control of the physical addition of the concentrate to the milk and (3) Control of the potency of the finished vitamin D milk.

The manufacturer of the concentrate must make certain that the potency of the concentrate which he sells to the milk industry is as claimed on the label, plus a reasonable safety factor.

Vitamin D concentrate should be supplied to the milk plant in sterile cans. Proper handling of these cans and the addition of the contents to the milk is of extreme importance, and if properly done the correct potency of the resulting vitamin D milk is automatically achieved.

If the first two steps are properly carried out the milk distributor need not worry about phase No. 3. J.C.M.

907. **Irradiation and Control of Vitamin D in Milk.** K. G. WECKEL, Univ. Wisconsin, Madison, Wis. Food Indus., 13, No. 6: 43-46. 1941.

The amount of vitamin D occurring naturally in milk is between 20 and 30 U.S.P. units per quart. Optimum potency desired is estimated to be between 135-400 units per quart. Therefore, the increase desired in an irradiated milk product is between 6 and 16 times the natural content.

The process of irradiating milk consists in exposing milk in thin films to radiation of wave lengths of from 2300 to 3100 Angstrom units. Several factors affect the response of milk to radiation; they are: (1) Characteristics of the film; (2) Characteristics of radiation; (3) Concentration of activatable substances and opacity of the milk to radiation; (4) Mechanical principles.

The method of creating the films in commercial installations consists of flowing milk by gravity over vertical, smooth, plane or cylindrical surfaces. The flow capacity of the film normally used ranges from 35 to 1,000 lbs. per linear foot per hour.

Radiation is derived from one of two sources: (1) the carbon electrode arc, or (2) the quartz mercury-vapor arc. With both types of arcs the power supply must be kept at a constant to insure proper radiation.

Normally, the variations in the fat content do not affect measurably the response of milk to radiation. It has been shown that significant increase in fat content of milk results in an increase (not proportionate) of the vitamin D content of the milk.

A number of modified milk products may be irradiated (*i.e.*, cream, evaporated milk, milk powder, and ice cream mix).

It is technically possible to irradiate fluid whole milk at almost any stage from the time of its production until filled into the bottle. Usually milk is irradiated just prior to its pasteurization. Evaporated milk is usually irradiated while hot when drawn from the vacuum pan and prior to storage.

The vitamin D of natural or fortified milk products is quite stable. It is stable at ordinary pasteurization temperature of 62.8° C. for 30 minutes or sterilization at 115.6° C. for 15 minutes. The form vitamin D₂ has been reported unstable at higher temperatures such as 232° to 260° C. for five minutes.

Two significant developments have resulted from research in the process of irradiation of milk during the past eight years. These are the increase in

flow capacities of commercial milk irradiators from 4,000 to 15,000 lbs. of milk per hour, and the increase of potency from 135 to 400 U.S.P. units per quart.

J.C.M.

908. **How to Add and Control Vitamin A in Margarine.** H. W. VAHL-TEICH, The Best Foods, Inc., Bayonne, N. J. Food Indus., 13, No. 6: 39-41. 1941.

The adding of vitamin A to margarine is a task which demands close contact between the laboratory and the purchaser. In the case of vitamin A the U.S.P. XI bio-assay method is the only one official in this country, but one cannot rely on it alone because it is too time consuming.

There must, therefore, be available more rapid and reliable day-to-day control tests. For this the Carr-Price quantitative test is used with the aid of a spectrophotometer. The control procedure is essentially as follows: (1) Determining of vitamin A potency of the vitamin A enriching oil to be used in margarine by the U.S.P. XI bio-assay technique. (2) Checking the vitamin A potency of the enriching oil with the quartz spectrophotometer based on the U.S.P. XI Standard of Reference Cod Liver Oil. (3) Determination of the presence of vitamin A in the margarine oil by the Carr-Price test. (4) Determination of the presence of vitamin A in the margarine oil by the quartz spectrophotometer. (5) Having the finished product checked by independent laboratories thoroughly familiar with the U.S.P. XI bio-testing procedure.

J.C.M.

909. **Defining Quantity Control.** ANONYMOUS. Food Indus., 13, No. 6: 37. 1941.

There are many in the food industry who see in the vitamin-enrichment program nothing more than an opportunity to sell more foods.

Quantity control does not, as many believe, imply making the best possible product, or putting in the most vitamins. The quantity level may be whatever management or government regulations define. In controlling quantity, the problem is to keep it uniform at whatever level of quantity has been selected.

Quantity control means the procedure of producing uniformity of quality by whatever means it may be necessary to employ.

J.C.M.

910. **How to Get Vitamin D into Milk by Feeding Cows Yeast.** CHARLES N. FREY, Fleischmann Lab., Standard Brands, Inc., New York, N. Y. Food Indus., 13, No. 6: 46-48. 1941.

Vitamin D was identified as a definite entity in 1922, the production of vitamin D by irradiation was established in 1924. The irradiation of yeast was begun on a commercial scale in 1928. The first yeast produced commer-

cially had a potency of 810 U.S.P. units. Today yeasts containing 7,200 U.S.P. units per gram are on the market.

Scientific investigations have shown that the production of so-called "metabolized vitamin D milk" of standard potency depends on three rules: (1) The amount of irradiated yeast to be fed depends upon the milk production of the cow; (2) The high producers are more efficient than the low producers in transferring vitamin D from the ration to the milk; (3) The yeast must be fed two or more times daily rather than at one feeding.

The milk reaches its maximum vitamin D potency after two to three weeks of yeast feeding. After the maximum is reached the potency will not change.

This article also tells how the potency of vitamin D is determined.

J.C.M.

911. Conference Sets Course to Better National Nutrition. GEORGE E. DOYLING, JR., Food Indus., Washington, D. C. Food Indus., 13, No. 7: 64-66. 1941.

On May 26-28 the National Defense Nutrition Conference held a meeting in Washington, D. C. Some of the topics discussed were as follows:

Recommended diets were set up with special stress on the fact that cheaper foods, such as inexpensive meat cuts and oleomargarine, are just as satisfying from the nutrition standpoint as the more expensive foods.

Fortification, restoration, and enrichment should be practiced only when absolutely necessary. Flour and bread are perfect examples where enrichment is necessary.

Above all others one point was stressed—education. All sides agreed that the front-line job is "slugging away," constantly using all the facilities for reaching public opinion that are available.

J.C.M.

912. How the American Soldier Is Fed. COL. PAUL P. LOGAN, Office of the Quartermaster General, Washington, D. C. Milk Dealer, 30, No. 9: 98-101. June, 1941.

The standard "garrison" ration is used in peacetime and under ordinary conditions for feeding soldiers in camps. This ration specifies (among other things) one ounce of evaporated milk, two ounces of butter, and one-quarter ounce cheese. The specified quantities are used only as a guide, and wide discretion is allowed to the post quartermaster and mess sergeant in actual purchase of food and serving of meals. The standard ration is not actually issued in kind. It is used as a monetary basis for the supply of subsistence or food.

Methods of purchasing, field rations, and the securing of fluid milk are discussed.

C.J.B.

ICE CREAM

913. Report of Committee on Sanitary Control of Ice Cream. F. W. FABIAN, Michigan State College, East Lansing, Mich. Jour. Milk Technol., 4, No. 2: 100. 1941.

Progress in the field of ice cream and frozen desserts is reported.

The report covers points relative to ice cream sanitation as experienced in several sections of the United States and Canada.

Regulation governing counter freezer installations and packaging ice cream receives considerable attention.

A good digest of Canada's legislation affecting packaging and selling of ice cream is given. L.H.B.

914. The Use of Corn Sugar Solids in Ice Cream and Ices. L. R. GLAZIER AND M. J. MACK, Massachusetts State College, Amherst, Mass. Canad. Dairy and Ice Cream Jour., 20, No. 5: 42. 1941.

A majority of consumers could discern no decrease in sweetness in ice cream when 25 per cent of the sucrose was replaced with corn syrup solids. There was a noticeable improvement in body and texture when this substitution took place. The use of corn syrup solids had little effect on titratable acidity and protein stability, raised the freezing point slightly, increased the mix viscosity about 10 per cent and had little effect on whipping ability. The development of sandiness on prolonged storage was delayed by the use of corn syrup solids. O.F.G.

915. Concentrating by Freezing to Protect Flavor. ANONYMOUS. Food Indus., 13, No. 4: 50. 1941.

To concentrate a solution, the solvent must be made to change its physical state so that it may be removed. One way is to convert it into a vapor by raising its temperature. In so doing the high temperature often injures the flavor of the liquid, or sometimes the flavor boils off also.

If reduced temperatures are used, the solvent can be partially frozen and removed from the solution by mechanical means. This principle depends on the fact that slow freezing of a solution (not eutectic mixture) will result in separation of crystals of the pure solvent.

Wilpert A. Heyman developed a machine of continuous concentration by continuous freezing. After a demonstration it produced a concentrate of about 50 per cent of its original volume by one passage through the cycle.

The apparatus consists of an inclined jacketed tube in which a helical worm rotates. Brine at -10° to -15° F. is fed into the jacket. Juice to be concentrated enters the space between worm and jacket, and as it moves it is frozen very slowly into a slush at 25° F. This slush is put into a perforated basket centrifugal. The concentrate is spun off leaving a white, tasteless water ice amounting to 50 per cent of the input juice.

A second passage will increase the total removal to 65 per cent or even higher.

In essence, this method of concentration depends on slow freezing as contrasted to quick freezing. Judging by the results of this demonstration it appears that continuous freezing for concentration must be done very slowly.

J.C.M.

916. **Corn Sirup Solids Improve Frozen Dairy Products.** LYNN R. GLAZIER AND MERRILL J. MACK, State College, Amherst, Mass. Food Indus., 13, No. 16: 68-70. 1941.

Added sweetening agents make up a large percentage of the food solids of frozen dairy products. They comprise about 40 per cent of the solids of ice cream.

Corn sirup solids result from the dehydration of corn sirup to a stable, white product. The carbohydrates presented in corn sirup solids are dextrose, maltose and dextrins.

The two principle objectives of this investigation were to determine the effect of corn sirup solids on flavor, body and texture of frozen desserts, and determine relative sweetening value of the common corn sweeteners when used in ice cream.

It was found that the viscosity of the ice cream mix increased about 10 per cent when corn sirup solids replaced 33 $\frac{1}{3}$ per cent of the sucrose.

An analysis of the consumer judgment leads to the conclusion that the replacement of 20 to 26 per cent of the sucrose improves the flavor, body and texture of ice cream, with no significant change in sweetness.

J.C.M.

917. **How to Make Naturally Flavored Maraschino-type Cherries.** F. A. LEE, N. Y. Agr. Expt. Sta., Geneva, N. Y. and E. A. BEAVENS, U. S. Dept. Agr., Geneva, N. Y. Food Indus., 13, No. 7: 52-54. 1941.

Cherries as gathered from the orchard cannot be processed into the colored product and still retain their natural color and flavor.

In these experiments Napoleon (Royal Ann) cherries were used throughout. In preparing naturally flavored cherries having the required firmness and color it was necessary to bleach and dye the fruit in the usual manner.

This article goes into detail telling how the sirup from Montmorency and English Morello cherries was extracted and added in different combinations to the bleached and dyed cherries to produce the best natural flavor.

J.C.M.

918. **Flash Sterilization Kills Spoilage Spores.** D. J. WESSEL AND H. A. BENJAMIN, American Can Co., Maywood, Ill. Food Indus., 13, No. 8: 40-43. 1941.

In the past years tomato juice canners have reported a certain amount

of spoilage, by microorganisms possessing resistance to destruction by heat.

This article tells of the trouble encountered in trying to kill these microorganisms without spoiling the flavor and general qualities of the juice.

The following process was found to kill the organisms and have no bad effect on the flavor. (1) Rapidly heat to 252° F. (96 seconds). (2) Rapidly cool to 190° F. and put into cans. (3) Put filled cans in boiling water for 16 minutes.

With the above procedure extreme care must be taken so that the juice will not become re-infected after the "flash" heating.

The problem presents suggestions to aid in handling dairy foods when spoilage organisms of this type present themselves. J.C.M.

Note: These organisms originating in the soil are not affected by temperatures commonly employed in heat-treating milk and dairy foods.

919. **Salaries, Seasons and Ice Cream Sales.** ANONYMOUS. Food Indus., 13, No. 8: 54, 91. 1941.

Consumption of principal manufactured dairy products has steadily increased since 1930. The trend in ice cream has been upward but not steady. In 1933 ice cream purchases were only two-thirds of 1924-29 annual sales.

The reason for this fluctuation in sales is caused by variations in the purchasing power of the urban population. Likewise, the individual consumption varies with the income level.

Regular temperature fluctuations, occurring during the year, naturally cause seasonal changes in ice cream consumption. No authentic data are available on these variations.

Ice cream production has become more uniform throughout the year. Probably education, promotion, and advertising had a great deal to do with the increase in winter months. The item of ice cream as a food has also been a factor in this development. J.C.M.

920. **Ice Cream Manufacture in 1941.** L. J. HYNES. Food Mfr., 13, No. 3: 60-61. 1941.

Ice cream is composed of substances which will be the first to be rationed when rationing starts, because it is regarded more or less of a "luxury."

There is an order prohibiting the use of liquid full-cream milk or skim-milk in ice cream. It has not affected the manufacturers very seriously, because most of them use milk solids for their mix.

The milk powders are also becoming limited. This article suggests such substitutes as unsalted margarine, and vegetable oils for butterfat; corn starch, and soya-bean flour for milk solids not fat, and honey for sweetening. J.C.M.

921. **English Fruit Juices as Source of Vitamin C.** VERNON L. CHARLEY, Long Ashton, Bristol, England. *Food Mfr.*, 16, No. 5: 102, 103. 1941.

The past six years have seen a steady development in the English fruit juice industry. The University of Bristol Research Station is making a study of fruit juices with regard to vitamin content. They tested the juices fresh and then after being canned from one to two years.

In apple juice it was found that canning reduced the already small vitamin content to practically nil. Strawberry juice for ice cream and other purposes decreases in vitamin content and flavor on storage and therefore is not satisfactory. Black currant juice can be sweetened and stored without serious losses of vitamin content. J.C.M.

922. **Selection and Use of Color in Ice Cream.** C. A. IVERSON, Iowa State College, Ames, Iowa. *Canad. Dairy and Ice Cream Jour.*, 20, No. 7: 25. 1941.

Desirable color increases the anticipated pleasure in eating food. The shade and intensity of color should harmonize with the product to be sold. Color should be uniform and free from flavor defects. A coloring material which changes its shade or intensity with varying acidity is undesirable. Highly colored ice creams are meeting with less and less favor by the consuming public. The author is not enthusiastic about vitamin fortification of ice cream in general but feels that the use of carotene concentrates may have merit. The cost of color purchased in dry form is lowest but keeping quality is usually best in paste form. A disadvantage in the purchase of liquid colors is the danger of considerable bacterial contamination. Boiling hot water added to the dry color to make the solution will give a liquid color which will keep for 2 weeks without danger of serious contamination.

O.F.G.

923. **Some Observations of the Vanilla Market.** R. SCHLOTTERER, Secretary, Vanilla Bean Assoc. *Canad. Dairy and Ice Cream Jour.*, 20, No. 7: 62. 1941.

Importation of vanilla beans has been drastically curtailed because of the European conflict. A continuance of the blockade of Madagascar will cause a lengthening of the vanilla famine since about 60 per cent of our beans have come from this and neighboring islands. It is estimated that the importation for 1941 will be about 20 per cent below average. Sources of supplies closer to our shores are Mexico, Tahiti and the West Indies. The quality of the Mexican crop is below par due to early picking of immature beans. Reliable reports about the 1942 Mexican crop are not encouraging. It is expected that about 200,000 pounds of Tahiti beans will be imported in 1941. Vanilla prices will continue to be high. O.F.G.

924. **A Study of Chocolate Coatings for Ice Cream Bars.** J. H. ERB, Ohio State Univ., Columbus, O. *Canad. Dairy and Ice Cream Jour.*, 20, No. 4: 68. 1941.

"Free fat" in the chocolate is responsible for the fluidity of the mass when heated to a temperature above the melting point of the fat. Other portions of the chocolate reduce the fluidity unless coated with a layer of fat. Factors which affect the coverage of chocolate are: (1) Composition of the coating, (2) Fineness of grinding, (3) Heat treatment when melting, (4) Temperature of the coating as well as the ice cream, when dipping, (5) Amount of moisture incorporated into the melted chocolate at the dipping tank, and (6) The use of lecithin. Lecithin retards thickening caused by excess moisture incorporation, by lowering the interfacial tension of the fat. The "bob" test can be used to determine very accurately the coverage properties of a coating. O.F.G.

925. **Selecting, Preparing and Using Flavors for Ice Cream.** E. G. WEED, Jackson, Mich. *Canad. Dairy and Ice Cream Jour.*, 20, No. 5: 58. 1941.

The most common causes of bad flavors in ice cream mixes are: (1) Sour, old or bitter cream, (2) Cream with oily, metallic, weedy or burnt taste, (3) Inferior quality condensed milk, (4) Inferior gum or gelatin, (5) Inferior fruits or nuts, and (6) Too much or not enough sugar. Covering up any of these undesirable flavors with fine flavoring produced no good results, especially with vanilla. The selection of flavoring is governed by a desire for quality and the need for economy. The author gives the following advice: (1) Do not guess when using flavor, (2) Measure accurately the amount of flavor used, (3) Confide in the supplier of flavoring, (4) Flavor makes or loses repeat sales, and (5) If flavor is worthwhile, then use the best. O.F.G.

926. **"Wave" Flavors for Ice Cream.** J. SHEURING, Univ. Illinois, Urbana, Ill. *Canad. Dairy and Ice Cream Jour.*, 20, No. 6: 18. 1941.

"Wave" or "ribbon" ice cream has come into prominence in the last two years and consequently has developed problems of manufacture. Settling of the "wave" flavor may be avoided by (1) having the ice cream as firm as possible consistent with good overrun as it leaves the freezer, (2) having as high viscosity in the flavoring syrups or gels as the pressure pump will handle satisfactorily, (3) having the flavoring material cold when added to the ice cream, (4) hardening the ice cream as rapidly as possible, and (5) maintaining the temperature of the distributing cabinets at a low point. Iciness may be avoided by (1) keeping the temperature of the flavoring material as low as possible without causing inconvenience in

handling, (2) keeping the concentration of the solids content of the flavoring material 40 per cent or above, and (3) maintaining the hardening temperature at a low temperature. Specifications and formulas are given for maple flavored syrups and gels, chocolate flavor, and fruit-flavored syrups.
O.F.G.

MILK

927. **Saving Dollars in Your Plant.** A. C. DAHLBERG, New York State Agr. Expt. Sta., Geneva, N. Y. *Milk Dealer*, 30, No. 9: 102-104. June, 1941.

Losses were determined in eight plants which received an average of 62,442 pounds of milk containing 2,148 pounds of butterfat. The average daily butterfat loss from the milk cans amounted to 2.5 pounds, which was approximately 0.1 per cent of all milk which was received. The total butterfat losses for the eight plants were 13.6 pounds per day. This is approximately 0.6 per cent of all the butterfat received. Figured on the basis of 25 cents per pound for butterfat, which is a very nominal cost, the total daily loss for New York State would amount to approximately \$5,000. The author gives suggestions for reducing these losses.
C.J.B.

928. **Some Experiments with Papaya in Milk.** J. H. NEWMARK, Pres., Merle Products Corp., Coconut Grove, Fla. *Milk Dealer*, 30, No. 10: 40-42. July, 1941.

Results of experiments over a period of time, using papaya pulp and juice (homogenized and vacuum packed) in milk, have indicated that papaya would be an important ingredient in infant feeding (and for adults, too), especially where there is an acid curd vomiting or digestive disturbance in the assimilation of milk.
C.J.B.

929. **Refrigeration Requirements in the Processing and Marketing of Milk.** KENNETH M. RENNER, Dept. Dairy Mfrs., Texas Technol. College. *Milk Dealer*, 30, No. 10: 44-48. July, 1941.

A brief summary of experimental work showing the rapidity and efficiency of cooling milk and a comparison of ice refrigeration and mechanical refrigeration, especially with reference to cooling costs, is presented.
C.J.B.

930. **New Developments in Milk Bottles.** F. P. GASS, Glass Container Assoc. *Milk Dealer*, 30, No. 10: 78-86. July, 1941.

A discussion of the ways in which the glass milk bottle is keeping the pace of progress in the dairy industry is presented.
C.J.B.

931. Report of Committee on "Chocolate Milk." SARAH VANCE DUGAN, Louisville, Ky. Jour. Milk Technol., 4, No. 2: 72. 1941.

Fourteen states reported that they have no standard law or regulation covering chocolate milk or chocolate drinks.

The laws vary for the other states, and these variations are given.

Inquiry was made of 30 manufacturers as to the composition of the products sold to milk plants for making "chocolate milk" or "chocolate drinks."

Reports regarding plant practices were obtained from 98 plants which were selling a "chocolate milk."

Four of the recommendations made by the committee are that "serious consideration should be given by states and municipalities to the need of more realistic definition of the product known as "chocolate milk" or "Chocolate Dairy Drink."

"The butterfat content of chocolate flavored milks should be determined by the state or local standard for whole milk reduced only by the addition of the syrup or powder."

"The bacterial standard of chocolate flavored milk products should be determined by the bacterial standards for milk." L.H.B.

932. Phosphatase Production in Dairy Products by Microorganism. B. W. HAMMER AND H. C. OLSON, Iowa State College, Ames, Iowa. Jour. Milk Technol., 4, No. 2: 83. 1941.

Using the modified Scharer phosphatase test (short test) it was found that various organisms produce phosphatase in sterile milk. Some of those belonging to the genus *Pseudomonas* were the most active, these include *Ps. putrefaciens*, *Ps. nigrifaciens*, and *Ps. mephitica*.

In the *Escherichia*-*Aerobacter* group, certain cultures of the *Aerobacter* group gave positive reactions while the *Escherichia* cultures were negative.

Five cultures of *Oospora lactis* gave positive reactions.

All of the *Streptococcus* or *Lactobacillus* groups studied were negative.

Several other groups were also studied, most of which were negative.

No detailed study was made of the number of organisms required in a product to give a positive phosphatase value.

The organisms producing phosphatase in milk, also produced it in butter, both salted and unsalted; but production was slower in the salted.

In applying the phosphatase test to butter, consideration should be given to the fact that phosphatase production by organisms in butter during the relatively long, normal holding period is possible. An important point in this connection is the ability of various *Pseudomonas* organisms to grow at relatively low temperatures.

From a limited number of trials made with pasteurized milk cheddar cheese that were still negative after several months it was assumed that less

danger of phosphatase production was possible in cheese than in butter, due to the fact that most of the active phosphatase producing organisms encountered are sensitive to acid, and therefore would be less likely to grow in sufficient numbers to produce a positive test.

L.H.B.

933. Report of the Committee on Education and Training. H. E. MILLER. Jour. Milk Technol., 4, No. 2: 87. 1941.

Most of the older members of the International Association of Milk Sanitarians obtained their training from personal experiences. Now that milk sanitation and quality control is rapidly progressing, the demand for milk sanitarians is greater than the supply, and there is an appeal to educational institutions to supply them. However, there is a limit to the number of positions available, so that it would be impractical to establish a full academic course in milk sanitation. The tendency is for schools of veterinary medicine, sanitary engineering, public health, dairy husbandry or dairy technology to provide courses in the more specialized phases of milk sanitation.

Although there is an increasing number of men entering the "profession" as milk sanitarians who hold degrees, there is still a large proportion who do not. They also believe that tests should be standardized in order that methods used for collecting samples and the interpretations of the results be uniform. To this end they recommend a committee be appointed by the International Association of Milk Sanitarians to cooperate with a committee of the American Public Health Association.

L.H.B.

934. The Phosphatase Test in Canada. C. K. JOHNS, Ottawa, Can. Jour. Milk Technol., 4, No. 2: 98. 1941.

A tabulation of the use of the phosphatase test in Canada is given.

L.H.B.

935. Contribution a l'étude des emplois des aciers inoxydables dans les laiteries et les fromageries. (Contribution to the Study of Uses for Stainless Steel in Dairies and Cheese Factories.) J. LEMOINE. Le Lait, 20, Nos. 198, 199, 200: 528-531. 1940.

The common uses to which stainless steel is put in milk processing plants and cheese factories are listed. The mechanical and physical properties of 8-18 stainless steel are described and its ability to resist the action of organic acids, detergents and sterilizing agents pointed out. Stainless steel containers for milk preserved by the Hofius process are almost essential.

O.R.I.

936. The Reduction of Resazurin in Milk and Aqueous Solutions. H. R. THORNTON, F. MCCLURE AND R. B. SANDIN. Univ. Alberta, Edmonton, Can. Canad. Jour. Res., 19, Sec. B. 2: 39-48. 1941.

Resazurin and methylene blue were compared in milks in which the oxi-

dation-reduction potentials were also electro-metrically determined. In aqueous solutions at pH 1 resazurin gave a perfect two-step titration curve and was reversible. At pH 7 the dye gave evidence of decomposing.

The blue-red reaction is electropositive and the red-white reaction electro-negative to the methylene-blue-methylene-white reaction. In milk the red-white reaction appears to poise the system rather strongly at that E_h level. The color attained at the end of one hour with resazurin in good quality milk depends upon the E_h set up by the dye and the milk and there is no evidence that the color developed is related to the number of bacteria in the milk. In poorer quality milks the test may have greater value. It is concluded that the action of resazurin in milk is not fully understood. O.R.I.

937. **The Value of Food Inspection and Properly Trained Food Inspectors.** A. J. SLACK, Univ. Western Ontario, London, Can. *Canad. Pub. Health Jour.*, 32, No. 7: 357-361. 1941.

Foods of animal origin are the chief field for the food inspector. Meat inspection requires fundamental training in anatomy, parasitology and bacteriology. Milk inspectors must be competent to interpret tests for herd disease in addition to being able to carry out most of the tests for milk quality. The food inspector of the future will be a liaison officer between the producer, retailer and medical health officer. O.R.I.

938. **Pasteurization of Skim Milk and Whey by Direct Steam Injection.** A. T. R. MATTICK AND W. A. HOY, Univ. Reading, England. *Food Mfr.*, 16, No. 5: 116. 1941.

A series of tests has been made to determine the bactericidal efficiency of an adaptation of the plate type heat exchanger for pasteurization of dairy by-products before return to the farm for stock feeding.

The principle of the new process is the direct injection of steam into the product as it leaves the heat exchanger. In treating milk or milk products for animal consumption the objects are similar to those for humans—there must be a maximum destruction of organisms causing spoilage, and minimum damage to the total nutritive value.

It is suggested to heat the milk to 175° F. and hold three seconds. This is sufficient to kill the general flora, including coliform organisms and *M. tuberculosis*. J.C.M.

939. **The Cause and Prevention of Milkstone Formation.** L. SHERE, Diversey Corp., Chicago, Ill. *Canad. Dairy and Ice Cream Jour.*, 20, No. 7: 22. 1941.

The amount of milkstone deposited, and its physical and chemical characteristics, depend upon such variables as the rate of flow, the rate of heating

or cooling, the amount of milk processed over a specific area of equipment, the composition of milk, the efficiency of everyday clean-up operations, the hardness of water, the water softening action of cleaners and sterilizers and the condition of the surface in contact with the milk. The major objections to milkstone are: (1) A source of high bacteria counts, (2) It is a good insulator of heat, (3) It may cause off-tastes and flavor, and (4) It is unsightly in appearance. Milkstone control consists of proper steps to minimize its formation and proper steps to remove its deposits. Steps for minimizing milkstone deposits are: (1) Rinsing with cold water after handling, (2) Thorough everyday cleaning, and (3) Sterilization with chlorine rather than heat. The method of removal to be used is dependent upon the type of milkstone; *i.e.*, whether it is due to a long series of deposits one piled on the other or whether it is due to a single day's operation. O.F.G.

940. **It's the Voice of Milk.** R. W. BROWN, Univ. Manitoba, Winnipeg, Man. *Canad. Dairy and Ice Cream Jour.*, 20, No. 7: 25. 1941.

This is a general article which discusses milk flavor (the voice of milk). According to the author abnormal flavors and odors in milk may be due to one or more of 5 causes which he lists as follows: (1) Deranged physical condition of the cow, (2) Consumption by the cow shortly before milking of strong-flavored feeds or weeds, (3) Absorption by the milk of odors, (4) Direct contamination of the milk with flavor-producing substances, and (5) Changes brought about by microorganisms. Ways and means of ameliorating or eliminating the causes of abnormal flavors are discussed. O.F.G.

941. **Pasteurization and Milk By-Products.** K. G. WECKEL, Univ. Wisconsin, Madison, Wis. *Canad. Dairy and Ice Cream Jour.*, 20, No. 7: 34. 1941.

Pasteurization of milk by-products differs from the pasteurization of milk in several respects. Not only is sufficient heat applied to destroy pathogenic organisms, but frequently to accomplish other purposes such as: (1) Development of a desired flavor, (2) Development of a physical property of body or plasticity. (3) Elimination of undesirable organisms other than pathogenic types, (4) To favor the solution of some ingredients, (5) Improvement in color, and (6) Production of desired texture. There are circumstances, however, during which the use of unnecessary amounts of heat injures the quality of the product by interfering with: (1) Desired enzyme reactions, (2) Bacteriological growth in the heated product, (3) Physico-chemical properties such as coagulability or firmness, and (4) Flavor. The pasteurization of such products as cultured buttermilk, cultured sour cream, chocolate milk, bottled cream, process cheese, cheese spreads and ice cream mix is discussed in order to illustrate the points listed above. O.F.G.

942. Principles and Problems of Short Time Pasteurization. G. E. WERST, The Creamery Package Mfg. Co., Chicago, Ill. Canad. Dairy and Ice Cream Jour., 20, No. 7: 35. 1941.

The author describes the theory and operation of plate exchange heaters and the operation of temperature and flow controls. Continuous operation, without shutdown, gives the most satisfactory and economical results. Variation in temperature is no serious problem and maintenance of the exchange equipment is simple. The control system must be of the precision type.

O.F.G.

943. A Discussion of Homogenized Milk. L. K. CROWE, Univ. Nebr., Lincoln, Nebr. Canad. Dairy and Ice Cream Jour., 20, No. 4: 46. 1941.

This is an article which summarizes the present knowledge about homogenized milk.

O.F.G.

944. The Processing of Milk as a Health Problem. A. WILSON, Medical Health Officer, Saskatoon, Sask. Canad. Dairy and Ice Cream Jour., 20, No. 4: 60. 1941.

"Certified milk" failed to satisfy public requirements because it was too expensive and it did not always protect against diseases being spread to the consumer. A perfectly clean milk was not always a safe milk. Physicians and pediatricians are now advocating the boiling of pasteurized milk for infants and some invalids. The emphasis for protection of milk supplies is now placed on the processing of clean milk so as to destroy infection rather than trying to keep the infection out of raw milk by inspection. It is as much the duty of municipalities and governments to provide safe milk as it is to provide safe water. The author lists a number of things the fluid milk industry should do to compete with evaporated milk and points out that advertising has played an important role in placing evaporated milk in its present prominent position with the consumer.

O.F.G.

945. The Sanitary Management of the Milk Plant. H. TRANMAL, Wisconsin State Dept. Dairying, Madison, Wis. Canad. Dairy and Ice Cream Jour., 20, No. 5: 72. 1941.

For good cleaning of equipment the aim should be to keep the milk solids in soluble form or to make them so with the aid of washing powders, water and proper temperatures. Drying and heating of residues on surfaces should be avoided. The presence of milk stone indicates carelessness. A successful procedure for cleaning dairy utensils is as follows: (1) Thoroughly rinse and scrub with cold water, (2) Scrub with a warm slightly slippery washing solution, (3) Rinse in clear water, (4) Rack the utensils and (5)

Sterilize just before milking time with a chlorine solution. Special precautions should be taken in cleaning tinned copper equipment to prevent metallic and oxidized flavor in milk. O.F.G.

946. **Factors Influencing the Flavor of Milk.** P. F. SHARP, Cornell Univ., Ithaca, N. Y. *Canad. Dairy and Ice Cream Jour.*, 20, No. 6: 26. 1941.

This article is mainly a review of the present knowledge of milk flavors. It discusses briefly the art and accuracy of flavor judging, the types of flavors frequently found in milk and some of the chemical reactions which produce certain of these flavors. The influence of processing and handling on flavor and the importance of milk deaeration as a means of preserving vitamin C and prevention of oxidized flavor are discussed. Deaerated milk has been kept for 3 to 4 weeks and maintained a flavor score of 21 to 22.5 without loss of vitamin C. Vitamin C is stable in deaerated milk even when exposed to sunlight. O.F.G.

947. **A Visible Method of Recording Sedimentation on the Farm.** JOSEPH BURNS, Schwartz Mfg. Co., Two Rivers, Wis. *Milk Dealer*, 30, No. 9: 86-90. June, 1941.

In an attempt to bring about "dirt consciousness" in dairy farmers, eight manufactured-milk patrons were furnished with visible recorders for 12 spent filter discs. The filter discs were returned to the plant, where they were graded. The results were highly satisfying, as six of the eight patrons showed an improvement in the amount of sediment on the discs. This method is recommended as a means of improving the quality of milk. The farmer forms a habit of observing his spent filter discs and, as shown by the above results, will usually attempt to improve them. C.J.B.

948. **How to Measure Bulk Quantities of Liquids.** NEAL M. CARTER, Pacific Fisheries Expt. Sta., Prince Rupert, B. C. *Food Indus.*, 13, No. 4: 41-42, 89. 1941.

This article discusses ways to accurately determine the volume and weight of liquids in tanks of different shapes. Temperature, distortion of container, effect of separated solids, froth and suspended bubbles must be taken into consideration. The results apply well to milk and cream.

As a unit of capacity the present U. S. gallon is the old English Queen Anne wine gallon, whereas the present Imperial is defined as "... the volume of 10 pounds avoirdupois of pure water, as weighed in air against brass weights, the water and air being at the temperature of 62° F. and the barometer at 30 inches."

Considerable exactitude is demanded in the control of certain processes in modern industries in order that standards and uniformity of quality may be maintained. However, commercial transactions and technological processes involving liquids frequently call for the handling of quantities too great for convenient direct determination in terms of weight.

One method of determining the weight of a bulk quantity of liquid is to attach a pressure-measuring device to the bottom of a tank having vertical sides. The gage reading in lbs./sq. in., multiplied by the tanks horizontal cross sectional area in sq. in. gives the number of pounds of liquid in the tank irrespective of any expansion or contraction of the liquid. Appreciable congealing or accumulation of solids at the bottom of the tank, may lead to failure of this method.

The indirect method, expression of volume or capacity in terms of weight is more generally used. It consists of most or all the following steps: (1) Determination of volume, (2) Measurement of temperature of liquid, (3) Expression of volume under standard conditions, (4) Application of conversion factor to change volume to weight. Tables and examples are given.

J.C.M.

949. **The Past and Future of Dairying.** H. A. RUEHE, Univ. Illinois, Urbana, Ill. Jour. Milk Technol., 4, No. 2: 78. 1941.

A brief history of dairying in Illinois, origin of the Chicago Milk Shed, and of the cheese, butter and evaporated milk industry in the state is presented.

Comparisons are made with present conditions. Competitive development of dairying in other sections is discussed, as are trade barriers and changing age levels, and their probable effect on the future of dairying.

L.H.B.

PHYSIOLOGY

950. **Some Morphological and Functional Relationships of the Bovine Hypophysis.** L. O. GILMORE, W. E. PETERSEN AND A. T. RASMUSSEN, Minnesota Agr. Expt. Sta. Tech. Bul., 145. 55 pp.

An interesting account of original studies on the morphology and histology of the bovine hypophysis (pituitary). Data were obtained from 139 females and 62 males of known origin. Observations and conclusions were:

The development of the bovine hypophysis from the fetal stage to maturity may be expressed by the shape of a sigmoid curve.

At birth the hypophysis is about .45 gram for females and .5 gram for males, although the range is from .3 to .7 gram for females and .4 to .8 gram for males.

The gland increases rapidly in size for the first three to four years, after which there is a decreasing rate of increase. In males the rate of increase decreases less rapidly than in females.

While the period of greatest size increase in the hypophysis corresponds to the period of greatest increase in body size, the hypophysis increase is much slower, as shown in comparing the weights of both hypophysis and body for given periods, to their respective birth weights.

The weights of hypophyses from freemartins tend to be smaller than for normal females. In part, at least, this is accompanied by smaller body weights.

No relationship between the relative hypophysis weight and producing ability is evident.

The shape as well as the weight of the bovine hypophysis varies markedly.

Distinct differences in the proportional weight of the anterior lobe are found between groups of the bovine. Whether that is due to inheritance or age could not be determined from the data at hand.

The presence of a cone of Wulzen does not appear to be limited by breed or sex, or to any particular post natal age.

The intermediate lobe in the bovine is exceedingly wide compared to that in humans.

In addition to the three ordinarily described types of cells (acidophiles, basophiles, and chromophobes) in the anterior lobe, the acidophiles were divided into two classes—crimson and brick-red acidophiles.

The presence of the crimson acidophile appears to be associated with sexual activity as in other species.

Differential cell counts indicate that there may be some difference between the bovine and some other species (cat, pigeon, and possibly the human).

No castration effect on proportional cell distribution in cattle was noted, but too few animals are involved to make this certain.

No nerve fibers were found in the anterior lobe of one bovine gland, but they were very abundant in the neural lobe and plentiful in the *pars intermedia*.

The text is well supplemented with tables, graphs, and illustrations. There is an extensive review of the literature based on a bibliography of 89 references.

J.G.A.

951. The Transfer of Radioactive Sodium Across the Placenta of the Goat. HERBERT A. POHL, LOUIS B. FLEXNER AND ALFRED GELLHORN, Dept. Embryology, Carnegie Inst. of Washington, Baltimore, Md. Amer. Jour. Physiol., 134: 338-343. 1941.

The rates of placental transfer per unit weight of placenta have been

measured with Na^{24} from a gestation age of about nine weeks until term. There is a three- or four-fold increase in transfer rate from the ninth week to about the nineteenth and twentieth weeks of pregnancy.

The placenta of the goat belongs to the syndesmochorial group. Its rate of transfer of Na^{24} per gram of placenta is of the same order of magnitude as that of the endotheliochorial placenta of the cat at comparable stages of pregnancy.

As pregnancy advances, there is a tendency for the tissue layers of the cotyledonary areas to be reduced to a degree commensurate with the increased nutrient requirement of the fetus. D.E.

952. Cortilactin, The Lactation Factor of the Adrenal. HERBERT S. SPOAR, FRANK A. HARTMAN AND KATHARINE A. BROWNELL, Dept. Physiol., Ohio State Univ., Columbus, Ohio. Amer. Jour. Physiol., 134: 12-18. 1941.

A lactation factor was prepared from the adrenal which possessed about one-tenth of the potency of purified prolactin when tested by the crop gland method. The daily injection of 1 mgm. cortilactin preparation into an adrenalectomized rat maintained on cortin enabled her to lactate normally. D.E.

MISCELLANEOUS

953. Sweet Water Systems. ANONYMOUS. Milk Dealer, 30, No. 9: 124-125. June, 1941.

Milk dealers using sweet water systems are apparently in the minority, it is indicated by reports received from a selected group of distributors in 16 states. The question, "Are you using a sweet water system" brought a negative answer from 67.3 per cent of the dealers replying, while 32.7 per cent advised that they were at present using a sweet water system.

The questions: "What capacity are you using?" "What capacity would you require?" and "How many uses do you have for cold water in your plant?" brought a wide variation in replies. C.J.B.

954. Cathodic Control of Corrosion. R. W. WARNER, Univ. Texas, Austin, Texas. Ice and Refrig., 100, No. 5: 354. 1941.

After a discussion of the electrolytic theory of corrosion, the author suggests methods for the prevention of corrosion to ice making tanks, piping and ice cans. The results of experimental work in connection with the use of protection anodes are described. The system of protection requires that current be taken from the metal part being protected to an external source of voltage. It is connected to the negative side of this source. From the

positive terminal of the source current is taken to the artificial anode and back to the brine solution from which it flows into the parts being protected. The electrical connections are such that the part being protected is made more negative to the solution than it was before, so that there is a positive tendency for current to flow toward the metal. The magnitude of the voltage must be just enough to insure current flow from the artificial anode to the cans, coils, and tank. The anode will be eaten away and must be replaced at intervals. A zinc anode is found to be very satisfactory. Cost figures for this system are included. L.C.T.

955. **The Profitable Operation of Locker Plants.** F. J. MOHER, Editor, "Meat Merchandising," St. Louis, Mo. *Canad. Dairy and Ice Cream Jour.*, 20, No. 5: 62. 1941.

This is an inspirational article dealing with ways and means of obtaining and retaining business in the profitable operation of a cold storage locker plant. O.F.G.

956. **Walls and Ceilings from the Sanitary Standpoint.** L. C. THOMSEN, Univ. Wisconsin, Madison, Wis. *Canad. Dairy and Ice Cream Jour.*, 20, No. 5: 74. 1941.

Light colors are recommended for the ceilings and upper walls because the lighter colors reflect more light and create better visibility. Experts recommend lighting of approximately 2 watts per square foot of floor space. Paint is important from the standpoint of sanitation. Mold infected walls may be cleaned by washing with trisodium phosphate, drying, washing with chlorine solution and again drying. Special paints containing fungicides are now available. The use of titanium oxide and high strength lithopones minimizes discoloration in white paints. The chief cause of paint peeling is accumulation of moisture between the paint and wall. Tan or buff colored glazed tile or brick have appealed to dairymen because they are harder, give better wear, do not soil as readily, are easier on the eyes and cost less than white brick. O.F.G.

957. **Quick Frozen Foods in Lockers.** J. H. L. TRUSCOTT, Ontario Agr. College, Guelph, Ont. *Canad. Dairy and Ice Cream Jour.*, 20, No. 6: 74. 1941.

The size of ice crystals in the flesh of fruits, vegetables, meats, etc., is indirectly proportional to the rate of freezing. When the crystals are too large, the cells are punctured and torn and lose their contents later when they are thawed out. Rate of freezing depends largely on the size of the object to be frozen. Freezing directly in the locker itself is impractical. Processing of vegetables and fruits previous to freezing is important.

Water-tight packages are essential. It has been found that frozen fruits retain a large proportion of their vitamin C but variable results have been obtained with vegetables. Although all the facts are not known there are indications that: (1) properly frozen products retain food values at least as well as any other method of preservation, and (2) vitamin C losses may be considerable during a 4-month storage period at any temperature above zero. Bacteria, molds and yeasts are reduced in numbers by from 50 to 90 per cent by freezing.

O.F.G.

958. **Use of Concrete in Dairy Plant Construction.** HUGH R. ROBERTS, Portland Cement Assoc., Atlanta, Ga. Milk Dealer, 30, No. 9: 54-58. June, 1941.

A discussion is given of the role which concrete can play in dairy plant construction. The proper use of concrete in such construction is discussed.

C.J.B.

959. **Problems of Brine Control.** FRANK PHILABERT, Chemical Solvent Co., Birmingham, Ala. Ice and Refrig., 100, No. 3: 203. 1941.

There are two steps in the handling of brine, (1) its preparation and (2) its maintenance. For its preparation a soft water free from silt and suspended matter should be used. A water which is good enough for drinking or ice making is suitable.

When calcium chloride is dissolved in water it is ionized into calcium and chlorine ions. The water reacts with these ions to form calcium hydroxide and hydrochloric acid. The reaction, however, immediately reverses itself. A mildly acid condition nevertheless is inclined to result. If foreign matter is present the equilibrium is upset and corrosion results. Hydrogen then is given off and dissolves in the brine, thus reducing the hydrogen ion concentration and making the brine more acidic. The addition of an alkali is a temporary remedy only, since it increases the concentration of foreign matter, and brings about side reactions. If chromates are used, the dichromate should first be converted into a chromate before use.

Ammonia leaks into brine are particularly bad from a corrosion standpoint. The ammonium hydroxide which forms, reacts with the calcium chloride to form ammonium chloride and calcium hydroxide. Since the calcium hydroxide settles out the brine is weakened. The ammonium chloride hydrolyzes and hydrochloric acid and additional ammonium hydroxide is formed. Again the brine becomes acidic unless ammonia continues to leak into the brine. In general the situation results in a very corrosive brine. The addition of hydrochloric acid to a brine which contains ammonia should be discouraged.

Brines should be checked periodically and properly treated when neces-

sary. Sediment should be removed by filtration, and the brine should always be maintained in a clear condition. L.C.T.

960. The Selection, Installation and Operation of Automatic Controls.

A. B. SCHELLENBERG, Alco Valve Co., St. Louis, Mo. Ice and Refrig., 100, No. 4: 284. 1941.

Advantages in the use of automatic controls for refrigeration systems are cited. Practical operating illustrations of their value are given. A number of automatic valves are described such as the thermostatic expansion valve, the multi-outlet valves, float valves, magnetic valves and evaporator pressure controls. Pictures and diagrams of these valves as well as installation diagrams are included. L.C.T.

961. Problems of Frozen Food Locker Plant Operation. ALFRED CORY.

Ft. Atkinson, Wis. Ice and Refrig., 100, No. 3: 243. No. 4: 313. No. 5: 389. No. 6: 463. 1941. 101, No. 1: 129. 1941.

This series of articles discusses the frozen food locker plant operation from a managerial standpoint. Emphasis throughout the series is on accounting, and forms are illustrated and methods described. The articles should be especially helpful to those who are just venturing into the frozen food locker business but they likewise contain excellent information for those with more experience. Some emphasis is placed on points to be observed in order to make the undertaking a successful one. L.C.T.

962. Safety in the Refrigeration Industry. JOHN M. ROCHE, Industrial

Safety Engineer, National Safety Council. Ice and Refrig., 100, No. 6: 420. 1941.

Various accidents and means for their prevention in the refrigeration industry are listed. Slips and falls are most often the result of the following unsafe conditions:

- (a) Defective or wet and slippery floors.
- (b) Improper illumination.
- (c) Defective stairs, ladders, ramps and scaffolds.
- (d) Running instead of walking.
- (e) Carrying loads or pushing trucks so as to obstruct vision ahead.

Strains and sprains resulting from industrial accidents indicate that the majority of them are due to the following:

- (a) Improper assignment of individuals predisposed to sprains and hernias to heavy manual labor.
- (b) Lifting too great a load unassisted.
- (c) Handling loads with poor footing.
- (d) Lifting too hastily, or carrying loads improperly.
- (e) Showing off.

Injuries due to handling tools were found to result from:

- (a) Using wrong tool for the job.
- (b) Defective tools.
- (c) Using defective tools such as shimming an oversize wrench.
- (d) Using correct tool improperly, such as pushing on a wrench or placing an extension handle on a wrench.
- (e) Using tools while in an unsafe position, or in such a manner as to create a hazard (using tools on pipes or apparatus under pressure).

L.C.T.

963. **A Democratic Industry under War Time Conditions.** A. L. GIBSON, Eastern Dairy School, Kemptville, Ont. *Canad. Dairy and Ice Cream Jour.*, 20, No. 4: 32. 1941.

This is a discussion of the part the dairy industry must play in war-time economy and the need for orderly planning of the whole industry so that it can be efficiently conducted under such conditions.

O.F.G.

964. **The Comparison of Odors.** H. D. RENNER. *Food Mfr.*, 16, No. 6: 131. 1941.

An apparatus has been devised which can be used as an aid in comparing intensities of odors. This apparatus was originally devised as a means of testing the olfactory powers of human beings.

The principal element of the apparatus consists of gas washing bottles made of brown glass, in which is placed the materials to be tasted. By means of bellows and a tube in the bottle air with a definite odor can reach the nose of the observer.

J.C.M.

965. **A Bacteriologic Study of a New Sanigenic Flooring.** W. L. MALLMAN, Mich. State College, East Lansing, Mich. *Jour. Amer. Med. Assoc.*, 117, No. 10: 844. 1941.

An investigation was made of the effect on microorganisms of using "hubbellite," a new cement floor surfacing material containing cupric oxychloride. The manufacturers claim that this flooring, when wet, releases a minute amount of a copper compound which exerts an oligodynamic action on bacteria in the water film on the floor surface. It was found that lethal effects were obtained when bacteria and molds were smeared on hubbellite tiles in the absence or presence of organic matter, and that under comparative conditions hubbellite flooring showed lower bacteria and mold counts than ordinary concrete floors.

D.P.G.

966. **Food Machinery for South America.** H. GORDON LAWSON JOHNSTON. *Food Mfr.*, 16, No. 4: 81. 1941.

During World War I the South American countries were growing rich from their sale of wheat, meat, and hides to Europe for the Allies.

After the war Argentine and Brazil started to study and develop the manufacture of food at home. The industry received full support of the government so it was soon a thriving condition.

This article tells how the branch agents should cooperate with the home office in selling of machinery and good will. J.C.M.

967. **The Control of Color in Food Processing.** H. W. RUDD, London, England. *Food Mfr.*, 16, No. 6: 129-130. 1941.

It is a known fact that the majority of people buy foods somewhat by their color. It is the job of the manufacturer to give the public what it desires.

The preservation of color during processing all types of foods comes under three heads: (1) changes occurring during the actual cooking; (2) changes during storage; (3) methods of assessing and standardizing color changes.

During storage color changes may be due to (1) natural metabolic processes, (2) interaction between food and material of container, (3) action of sunlight (usually results in fading). J.C.M.

968. **Nitrate Kills Odor in Waste.** N. H. SANDBORN, Natl. Canners Assoc., Washington, D. C. *Food Indus.*, 13, No. 4: 57-58, 101. 1941.

Studies on the nitrate treatment of lagooned wastes were not undertaken as the development of a new method for treating cannery wastes but rather as a corrective measure for those lagoons now in existence which are so located as to produce an odor nuisance.

The ability of nitrates to prevent putrescence in domestic sewage was recognized in 1892.

It is well known that organic matter in solution rapidly depletes the water of its oxygen content. When this condition is obtained the organic matter undergoes anaerobic decomposition with the production of offensive odors. By supplying an available source of oxygen the organic matter may be made to decompose without odor formation. Nitrates furnish such a supply of oxygen.

The following conclusions were drawn after tests were made with nitrate: (1) It is not practical to treat all wastes; (2) At a 40 per cent dosage rate satisfactory odor control is possible; (3) Partial odor control is possible with a 30 per cent dosage rate.

It is believed that the use of sodium nitrate at lower dosage rates starting at the time the disposal plant begins to operate would prove to be more effective. J.C.M.

969. **After the War—What of Agriculture?** R. McQUEEN, University of Manitoba, Winnipeg, Man. *Canad. Dairy and Ice Cream Jour.*, 20, No. 5: 36. 1941.

The first part of this article deals with the development and early eco-

conomic history of Canada up through the last World War to 1919. The problems and burdens of prosecuting the last World War are contrasted with those being encountered in carrying on the present conflict. The farmers of western Canada are faced with the problem of a great wheat surplus and consequently must change their crops or accept a decrease in standards of living. If, after the war, international trade revives, a change may not be necessary. If a change is necessary the author believes the raising of hogs and the production of dairy products possess the greatest comparative advantage next to wheat for western Canada. O.F.G.

970. **The Disposal of Dairy Wastes.** H. A. TREBLER, Sealtest Res. Lab., Baltimore, Md. Jour. Milk Technol., 4, No. 2: 93. 1941.

The essential thing is to cut down waste in the plant to the least possible amount. Small amounts of milk wastes are not detrimental, and may even be beneficial to healthy stream conditions providing the reaerating ability of the stream is great enough.

In some cases the amount of water in the stream used for disposal is great enough during a portion of the year so that treatment will not be necessary.

If complete treatment is necessary, it is recommended that one of the well-established firms which are now specializing in dairy waste disposal be consulted. L.H.B.

971. **Low Pressure Boilers for Dairy Plants.** DAVID BURKE, Elm Farm Dairy, Albany, N. Y. Milk Dealer, 30, No. 10: 35, 66-67. July, 1941.

The author discusses the following advantages of low-pressure boilers: Reduced labor costs, less space required, fully automatic, lower initial cost, and elimination of high-pressure steam leaks in pipe lines and the burning out of valve seats on valves used to control and throttle steam for hose lines.

C.J.B.

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